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Virus Diseases of Tobacco in Kentucky

A thesis presented to the faculty of the University of Minnesota in partial fulfilment of the requirements for the degree Doctor of Philosophy.

—BY—

E. M. JOHNSON

June 1930

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Agricultural Experiment Station

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VIRUS DISEASES OF TOBACCO IN KENTUCKY

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BULLETIN NO. 306

(RESEARCH BULLETIN)

Virus Diseases Of Tobacco In Kentucky

E. M. JOHNSON[1]

INTRODUCTION

The early literature seems to indicate that the belief was prevalent that mosaic was the only virus disease of tobacco. It is not unlikely that symptoms other than those typical for tobacco mosaic were observed and perhaps were considered as different manifestations of this disease. Following the investigations of Schultz and Folsom (25)[2] who described several virus diseases of the potato, a number of virus diseases of tobacco, differing in symptoms and properties from tobacco mosaic, were reported. Johnson (15) described 10 and Fernow (8) 8 virus diseases which affect tobacco. Fromme, Wingard and Priode (9) conclusively demonstrated the transmissibility of ringspot of tobacco, a disease which seems identical with the one earlier illustrated by Johnson (13) as a non-parasitic disease. Valleau (28) compared the disease to Liesegang rings, reported 2 cases of successful transfer, and suggested that it might be of a virus nature. This disease differs in symptoms and host range from the "ringspot" which Johnson (14) obtained from "healthy" potatoes. McKinney (17) described three mosaics capable of affecting tobacco, yellow, light-green, and mild dark-green, which he obtained from the Canary Islands from *Nicotiana glauca* plants. These three virus diseases which are readily transmissible to tobacco appear to be true tobacco mosaics in the sense used in this paper, because the dried tissue retained its infectivity for a year. Valleau and Johnson (32) described 10 virus diseases affecting tobacco, all

[1]The writer wishes to acknowledge the helpful suggestions given by Dr. W. D. Valleau thruout the course of the work and in the preparation of the manuscript.

[2]Numbers in parentheses refer to "Literature Cited."

from naturally infected plants. These and other virus diseases of tobacco will be discussed in this paper.

HISTORICAL REFERENCES

The first definite reference to a virus disease of plants was that of Mayer (16), in 1886, who described the "Mosaikkrankheit" of tobacco. The name refers to the pattern produced by the intermingling of chlorotic and normal green tissue which has some resemblance to the mosaic produced by inlaying tiles of different colors. Since Mayer's discovery of tobacco mosaic, a great many virus diseases have been described. According to Kunkel (24, Rivers, pages 345-348) at least 200 plant species are affected with virus diseases.

Until recently, the work on the plant viruses was confined almost entirely to the mosaic disease of tobacco as originally described by Mayer (16). It consisted in the demonstration of transmissibility, by Iwanowski (11); filterability, by Beijerinck (2) and by Duggar and Karrer (5); various properties of the virus, by Allard (1), and in attempts to assign the causal entity to enzymes, by Woods (37), to bacteria, by Iwanowski (11) and Mayer (16), to a toxin, by Hunger (12), and to protozoa, by Nelson (20) and others. None of the attempts to discover the etiological nature of the virus has been successful because no one has been able to conclusively demonstrate the cultivation of the virus in vitro. Olitsky (21) reported the successful cultivation of what he thought was the active agent of tobacco mosaic in vitro, but later work by Mulvania (19) and Purdy (22) did not verify Olitsky's assertion. The work of Purdy (23) in which she demonstrated that the virus of tobacco mosaic multiplies in detached leaves is at least suggestive of the living nature of this virus.

Kunkel (24, Rivers, pages 335-359) has given a good description of the present status of the virus problem and contributed a bibliography of the most important papers on this subject.

STATEMENT OF THE PROBLEM

Tobacco is the most important cash crop in Kentucky, and its profitableness depends upon the production of good yields of

high quality. The observation was made that one of the virus diseases had an adverse effect on the yield and quality of tobacco (30). It was thought desirable, therefore, to learn something of the prevalence of virus diseases in this plant as well as something of their host range. The work reported in this bulletin was done to obtain information on the prevalence, transmissibility and host range of the 10 virus diseases of tobacco, described by Valleau and Johnson (32), and 8 other virus diseases to which tobacco is susceptible. In addition to these 18 viruses, 2 mixed virus diseases were studied because, for a time, they were thought to be caused by a single virus.[3]

NOMENCLATURE

The term, tobacco mosaic, as used in this paper, refers to those diseases of tobacco, the infective agents of which withstand long periods of drying[4] and seem to affect only the solanaceae. The causes of the other diseases described are considered to be viruses because the extract from diseased green tissue is infectious and the chlorosis, hypoplasia, hypertrophy, and necrosis produced in affected plants are typical of virus diseases in general. The latter diseases are not classed with the tobacco mosaics because the infective agent is destroyed by drying (Table 2). The term, cucumber mosaic, is retained because of its wide use in the literature. That it is difficult to devise a satisfactory system of nomenclature for the virus diseases of any plant species is shown by the confusion which has arisen from the names used for the virus diseases of potatoes. As was suggested by Walker (35), there is no way of telling whether a virus disease, of tobacco for example, is original with that host or whether it is a virus disease of some other species, on tobacco. If, however, a virus disease is of frequent occurrence on a specific host in nature and produces characteristic symptoms that are constant for the host in question, there seems to be no great objection, for purposes of description, to speak of the disease as of this host. In this bulletin, the virus diseases of tobacco, except for the cucumber mosaics and the "healthy

[3]This is the reason why these mixed viruses were used in some of the studies reported in this bulletin.

[4]According to Valleau and Johnson (29) as long as 30 years, at ordinary temperature.

potato'' virus, are named after some prominent symptom on this host.

MATERIALS AND METHODS

Turkish tobacco was used in the greenhouse because it has small leaves and can be grown to maturity in restaurant tumblers and half-gallon jars, if fertile soil is used and liberal applications of nitrogen salts are made. The plant has an upright habit of growth and a small spread of leaves, which minimizes the danger of accidental infection by the rubbing together of the leaves of different plants. The virus diseases described in this bulletin produce the same symptoms on Turkish as on Burley tobacco. A fertile soil from a virgin forest was used and nitrate was applied liberally at frequent intervals.

Previous work at this Station had shown that the finger method of inoculation is unsafe, even tho the hands are thoroly washed with soap and water before and after each inoculation. Pricking was found to be an unsatisfactory method with some of the viruses. The sterile swab method, which was satisfactory, was used in the experiments unless some other method is indicated. One-third the length of a piece of wood 6 inches long and ¼ inch square was wrapped as smoothly as possible with 2 or 3 thicknesses of cheesecloth which was held on by a cord. This swab, following autoclaving at 15 pounds pressure for 15 minutes, served as a pestle for crushing the infected tissue, as well as for rubbing the latter on the plant. Sterilized culture dishes were used as mortars. Leaves intended for inoculum were removed from the infected plant by means of flamed forceps, and crushed in a little sterile water. A piece of waxed paper was placed on the palm of the hand and the hand thus protected was used as a support for the leaf to be inoculated. The swab was dipped into the crushed tissue and rubbed on the leaf, only sufficient pressure being applied to break the epidermis in several places. Rubbing without visibly wounding the epidermis was effective with the tobacco mosaics, but with some of the other viruses, infection was less certain. In this procedure the fingers do not touch either the plant used as inoculum or the one being inoculated.

Thruout these studies every precaution was taken to prevent accidental infection. Small numbers of plants were used

in most of the experiments, but because of the care used in inoculation, and other precautions, it is believed the results are comparable to those that would have been obtained with greater numbers.[5] It was believed that repeated inoculations on small numbers of plants at different times would be more valuable than a greater number made at one time.

In nearly all the experiments the jars or tumblers were sterilized before they were filled with soil. Fresh soil was used for each experiment because some earlier work indicated that soil in which virus-diseased plants had grown was unsafe for other work. It has been the rule thruout this work never to touch a plant with the hands. The plants were set with flamed forceps and scalpel. Greenhouse insects were kept under control by fumigating with hydrogen cyanide and by dusting the plants with nicotine-lime dust.

SOURCES AND DESCRIPTIONS OF THE VIRUSES

The descriptions which follow are based largely on the results of inoculations given in Tables 1 and 21.

Severe mosaic type 1. (Plate I, Figure 1 and Plate IV, Figure 2.) This virus was obtained October 22, 1925, from dried tobacco of the 1920 Experiment Station crop. This seems to be what Johnson (15) describes as tobacco virus 1. Severe mosaic type 1 produces prominent mottling, consisting of raised, dark-green, blister-like areas along the veins, and pale-green tissue between the veins. In addition to the mottling, the plant is stunted and the leaves are small and distorted. The new leaves that form after infection develops are usually narrow and pinched at the tips and their margins are frequently inrolled (Plate 1, Figure 1). Necrosis usually occurs on the lower leaves of plants affected in the field as irregular brown or parchment-like areas which range in size from ⅛ to several inches in diameter. When small and numerous, these spots give the plant a characteristic speckled appearance. The dead tissue may fall from the leaf, leaving only the larger veins. Symptoms appear in 3 to 15 days after inoculation with the extract from either fresh or dried material, the average incubation period being 2

[5]There have been fewer than 15 accidental infections in nearly 10,000 inoculations in the greenhouse where these studies were made.

to 5 days longer with extract from dried material than with that from fresh tissue. The first sign of infection, on vigorously growing plants, is a clearing of the veins of the new leaves, with or without rugoseness, or the appearance of small, pale-green spots in the vein islets. Local infection sometimes develops on rubbed leaves as small, circular, almost invisible spots $\frac{1}{8}$ to $\frac{3}{16}$ inch in diameter. If the light intensity is low, mottling is the first visible symptom. This type has not changed thruout 20 successive transfers in tobacco (Table 1) and its infectivity is not destroyed by drying at room temperature (Table 2).

Severe mosaic type 2. (Plate I, Figure 2.) This virus was obtained from Burley tobacco in Fayette county, Kentucky, on July 16, 1927. It produces fewer blister-like areas than type 1, but the areas are larger. Sometimes the leaves of affected plants are little more than midrib and the plants are always stunted more than by type 1. The incubation period is about the same as for type 1, but symptoms may be visible a day sooner. The first visible sign of infection is vein clearing accompanied by rugoseness of the twisted bud leaves. The dark-green areas along the veins appear 1 to 3 days later than in plants inoculated with type 1. No change in virulence was noted in 12 successive transfers in tobacco (Table 1) and the infectivity is retained in dried material (Table 2).

Mild mosaic type 1. (Plate 2, Figure 1.) This virus was obtained October 22, 1925, from cured tobacco of the 1920 Experiment Station crop and, like severe mosaic type 1, was 5 years old when the first transfer was made to tobacco plants. The most characteristic symptom is a mild mottling formed by the production of pale-green tissue between the veins and darker tissue along the veins. The dark areas along the veins are rarely blister-like and affected plants are stunted so slightly that usually it is not noticeable. Necrosis in field and greenhouse plants is slight. The first sign of infection is an indistinctly visible clearing of the veins of newly developing leaves with only a slight tendency to rugoseness. The symptoms were constant thruout 20 successive transfers in Turkish tobacco and were the same from either fresh or dried material (Tables 1 and 2).

Mild mosaic type 2. (Plate II, Figure 2.) This virus,

obtained from a naturally-infected Burley tobacco plant in Fayette County on July 19, 1927, is very similar to mild mosaic type 1. All the symptoms are the same except that in field-inoculated plants type 2 produces a more diffused type of chlorosis than type 1. These two mild types can be distinguished without difficulty when Burley plants are inoculated in the field, but in the greenhouse, both mild types produce symptoms that are so nearly identical that it is difficult and usually impossible to distinguish between them. Mild mosaic type 2 has retained its properties thruout 12 successive transfers in tobacco (Table 1) and drying has not destroyed its infectivity (Table 2).

The four mosaics described should be looked upon as types or strains which differ in degree rather than in kind. Any one of them could be considered as the disease commonly referred to in the literature as typical tobacco mosaic. The fact that they retain their respective characteristics thruout a number of successive transfers (Table 1) and drying does not change their characteristics indicates that they are enough unlike each other to be considered as types. The severity of injury to tobacco has been used as a basis of separation and it is not unlikely that more extensive inoculations from naturally-infected plants would reveal types that differ slightly from these four.

Yellow mosaic. (Plate III, Figures 1 and 2.) This virus was obtained from a naturally-infected Burley plant in Fayette County on July 30, 1927. It is readily distinguished from the mosaics previously described by the presence of prominent lemon-yellow tissue between the veins of younger leaves (Plate III, Figure 2). At times, however, especially during the winter, in the greenhouse, the yellow areas may not develop and the plants may appear similar to those affected with severe mosaic type 1. Dark green, blister-like areas occur along the veins and there is distortion and twisting of the bud leaves. Affected plants are markedly stunted. Liesegang ring-like patterns consisting of narrow, alternate bands of pale green and yellow tissue develop on leaves above rubbed ones in two to several weeks after inoculation (Plate III, Figure 1). The first symptoms after inoculation, either in the field or greenhouse, are vein clearing accompanied by rugoseness of the growing point leaves, followed by mottling. The yellow tissue usually does not

develop sooner than 7 to 10 days after mottling appears, but it may develop as the first symptom. The incubation period varies from 3 to 12 days. Necrosis occurs on older leaves in the field as irregularly outlined to circular brownish areas which frequently drop out so that affected plants have a ragged appearance. This yellow mosaic does not seem to be the one which McKinney (17) described in Connecticut-Havana tobacco and which he illustrated in Figures 5B and 7. If the yellow mosaic, described in the present paper, is a mixture of a green and a yellow mosaic, the latter appears to predominate both in the field and greenhouse, whereas in McKinney's mixture the green seems to predominate. Small, yellow spots like those described by McKinney have been found often in the green mosaics in Kentucky but separations have not been tried.

White mosaic. (Plate IV, Figure 1.) This virus was obtained from a naturally-infected bell pepper (*Capsicum frutescens var. grossum*) on the Experiment Farm on August 10, 1928. The pepper plant was severely distorted and dwarfed and the leaves were creamy-white, except for narrow bands of tissue along the veins. Tobacco plants affected with this virus gradually assume a creamy-white color in irregular areas between the veins. Narrow bands of blister-like tissue may develop along the veins or an occasional raised blister may appear between the veins (Plate IV, Figure 1). Sometimes the lower leaves die, so that in a few weeks little remains but a few small, almost white, malformed leaves at the growing point. The first symptom is the appearance of circular yellow spots $\frac{1}{16}$ to $\frac{1}{8}$ inch in diameter on rubbed leaves. Sometimes these spots increase in diameter and become necrotic or they may become almost white without becoming necrotic. White mosaic occurs rarely if at all as a distinct disease in Kentucky tobacco. It was tested in the field in 1929 (Table 21). Field plants, when inoculated, develop numerous circular, chlorotic areas on rubbed leaves which increase in size and become necrotic. Entire leaves sometimes are killed by the coalescing of these spots. Large, white streaks $\frac{1}{4}$ to $\frac{3}{4}$ inch in width and from $\frac{1}{4}$ to several inches in length, occur on the stem. These white areas become necrotic and gradually extend thru the tissues to the pith which may be slightly discolored. The stem lesions extend

into the midveins of the leaves, dying first being observed in the vascular tissue about an inch from the point where the leaf clasps the stem. Such leaves break at the necrotic area and hang down. Plants viewed at a distance resemble published photographs of tobacco affected with Granville wilt (26). The seed pods and the calyx tubes are mottled with pale green to white areas and never develop normally in plants affected in the field. The description which McKinney (17) gives of yellow mosaic in Connecticut-Havana tobacco resembles very closely the white mosaic described here. In McKinney's Figure 9 is illustrated the stem mottling which occurs with white mosaic, and a spotting of the seed pods similar to that which McKinney illustrates in Figure 10 has been observed. White mosaic appears to be a mixture of a white and a severe mosaic (green), as a transfer from the dark green blisters gave a severe mosaic with only small yellow spots. A difference may lie in the fact that green mosaic symptoms predominated in the case of McKinney's disease, whereas white mosaic symptoms appear to predominate in this mixture.

Ring mosaic. (Plate V, Figures 1 and 2, and Plate XIV, Figure 1.) The virus of this mosaic was obtained from a field of Burley tobacco in Fayette County on August 10, 1927. In addition to the mottling and distortion, which are similar to these symptoms in severe mosaic type 1, this mosaic produces definite rings consisting of alternate bands of pale green to yellow tissue. In greenhouse plants these rings are, for the most part, almost perfect circles (Plate XIV, Figure 1), whereas in field plants they are more frequently irregular spots (Plate IV, Figure 1). The circumference gradually becomes necrotic and this necrosis eventually involves the entire spot (Plate IV, Figure 2). The first symptoms that appear are like those in severe mosaic type 1. Rings may appear a week after mottling develops or they may not appear until the plants are near maturity. This type rarely occurs in the field in Kentucky. There has been no change in this type in 12 consecutive transfers in tobacco (Table 1), and its infectivity is not changed by drying at room temperature (Table 2).

Etch. (Plate VI, Figures 2A, 3, 4 and Plate VIII, Figure 1.) This virus was obtained from naturally-infected Burley

tobacco in Fayette County on September 16, 1926, and has been found many times since. It was also obtained from cigar tobacco collected near Miamisburg, Ohio, on August 19, 1927, and a strain almost but not quite the same was received from Thomasville, Georgia. The leaves of the growing point of affected plants have small chlorotic spots 1/16 to 1/4 inch in diameter, in the interveinal areas (Plate VI, Figure 3). The margins of these spots become either partly or entirely necrotic. The lower leaves of plants affected in the field develop necrotic veins which have an ochre-yellow to rusty-brown color, a symptom also typical of etch + and severe etch (Plate IX, Figures 1 and 2). The first sign of infection usually appears about 6 days after inoculation on rubbed leaves and the younger unrubbed ones, in the form of fine necrotic etching on the upper surfaces (Plate VI, Figure 4). A few days later, chlorotic spots appear in the bud leaves and these later become necrotic. Symptoms may continue to develop in this sequence for some time and then leaves that show only occasional chlorotic spots may develop for several weeks. If such plants are kept in a vigorously growing condition, for a sufficient time, numerous chlorotic and necrotic spots may again appear. Seventeen successive transfers were made in tobacco without changing the characteristics of this virus (Table 1). The infectivity is destroyed by drying the tissue at room temperature.

Etch (Georgia). This virus was sent to the writer by Mr. R. T. Henderson from Thomasville, Georgia, on July 16, 1928. It is similar to, altho not identical with, the Kentucky etch. It differs from the latter only in the production of slightly larger chlorotic spots and necrotic circles. The difference between the two types is so slight as to be noticeable only by direct comparison of plants inoculated with the two types at the same time.

Etch +. (Plate VII, Figure 1.) This virus disease so named because it was believed, at the time of collection, to be a mixture of etch and a mosaic, was obtained from a naturally-infected Burley plant on the Experiment Station Farm on August 11, 1927, and has been seen many times since. Inoculation from dried material gave no infection, which showed that tobacco mosaic was not present. In the greenhouse the

symptoms, including the development of spotting and etching, are similar to these symptoms in etch but are more prominent. The leaves of the growing point are rugose and distorted, a condition never observed in etch plants in the greenhouse. The upper leaves of field plants are narrow and chlorotic, and small, slightly elevated areas develop along the secondary veins, which give the leaves a peculiar appearance not unlike the grain on tanned alligator hide (Plate VII, Figure 1). A mosaic-like mottling may occur on plants in the field but such plants can be distinguished from mosaic plants by the etching, rusty-brown veins, and the so-called alligator hide effect. Eleven successive transfers (Table 1) were made in tobacco and no changes in the symptoms were observed.

Severe etch. (Plate VI, Figures 2B, Plate VII, Figure 2, and Plate IX, Figures 1 and 2.) This virus disease, which is of infrequent occurrence in the tobacco fields of Kentucky, was first obtained from Miamisburg, Ohio, on August 19, 1927. The symptoms are of the same general type as those of etch and etch +, except that they are in all particulars more prominent than either. The affected plants are stunted more and chlorosis and necrosis are more extensive. The bud leaves have a crinkled appearance, and in the field this is accompanied by a downward rolling of the leaf margins and a hooking back of the tips (Plate VII, Figure 2). Older leaves of plants affected in the field develop vein necrosis of the type described for etch and etch + on the midribs as well as on the veins (Plate IX, Figure 2). Patterns consisting of irregular to circular chlorotic areas bounded by necrotic tissue appear on the lower leaves of plants in the field (Plate IX, Figure 1). The first symptom is the development of etching on rubbed leaves in 3 to 6 days. Chlorotic spots appear in the wrinkled bud leaves a few days later. Severe etch has retained its characteristics thruout 14 consecutive transfers in tobacco (Table 1). The virus is destroyed by drying (Table 2).

Veinbanding. (Plate X, Figure 1.) This virus disease described as "vein margin" by Valleau and Johnson (32) was first obtained from a Burley plant on the Experiment Station Farm on July 23, 1927. The name, veinbanding, was suggested by the narrow bands of dark green tissue which appear along

the veins of the leaves of plants that have been infected for some time (Plate X, Figure 1). Sometimes these bands are visible only on the frill at the base of the leaf where it clasps the stem. Small, just-visible, chlorotic spots which give the plant a mottled appearance, sometimes develop between the larger veins of leaves of plants in the greenhouse and in the young leaves of field plants. The lower leaves of infected greenhouse plants sometimes droop. On the older leaves of field-infected plants chlorotic spots are sometimes found which may become quite large and might be confused with early symptoms of ringspot. Veinbanding is of frequent occurrence in and near vegetable and potato-growing areas and is readily obtained from mottled potatoes. This type has not changed its characteristics thruout 13 successive transfers in tobacco (Table 1). The infectivity is destroyed by drying at room temperature (Table 2).

Coarse etch. (Plate VIII, Figure 2, and Plate X, Figure 2.) This disease, which might be confused with a small-pattern ringspot or the "healthy potato" virus disease, was obtained from naturally-infected Burley tobacco on the Experiment Station Farm on June 24, 1927. It was observed in cigar tobacco in the Miami valley in Ohio, and is present in the dark tobacco section of Western Kentucky. The most prominent symptom is the coarse etching which appears usually as small necrotic circles and arcs of various sizes, the former sometimes being almost an inch in diameter (Plate VIII, Figure 2). These necrotic areas are always larger than in the etch virus diseases and usually smaller than in ringspot or at least with a more narrow necrotic border than in the latter. Leaves at the growing point develop beautiful chlorotic ring-and-line patterns between the veins, a symptom that may be used to distinguish coarse etch from ringspot (Plate X, Figure 2). These patterns develop necrosis after variable periods of time. In the greenhouse the first symptoms appear in 4 to 11 days as coarse etching in the form of single or concentric rings and lines on rubbed leaves. These are generally finer than those of ringspot. The chlorotic patterns may appear in the young leaves a few days later or not for several weeks. No change in this type has occurred in 14 successive transfers in tobacco (Table 1). The infectivity of coarse etch is destroyed by drying (Table 2).

Ringspot. (Plate VI, Figure 5, and Plate XI.) The ringspot disease described in this bulletin, identical with the one described by Fromme, Wingard and Priode (9), was obtained on September 16, 1926, in a tobacco plant-bed on the Experiment Station Farm. Since then an occasional ringspot plant has been found in plant-beds. Ringspot was transferred to tobacco from naturally-infected cucumber from 2 sources; one from a field of Burley in Fayette County which had 5.7% ringspot in the tobacco (Table 9, Farm 5) and where cucumbers were planted in the missing places in the tobacco rows, and the other from near Hardinsburg, Kentucky. Ringspot virus was transferred to tobacco from naturally-infected Cobbler potato foliage collected on June 6, 1928 (Table 9, Farm 1). The potato foliage had lemon-yellow areas either along the leaflet margins or between the veins. These areas form ring-like patterns (Plate VI, Figure 1). The tubers from this plant were planted in the spring of 1928 and the foliage again showed typical symptoms. Tobacco was inoculated and typical ringspot accompanied by the "healthy potato" virus disease developed (Plate VI, Figure 5). The ringspot virus, collected on September 16, 1926, was taken thru 8 successive transfers in tobacco and lost. The one listed in Table 1 is from the Hardinsburg cucumber and is the one used in the inoculations in Table 21, from which the photograph in Plate XI was taken. Besides the symptoms described by Fromme, Wingard and Priode (9) naturally and mechanically-inoculated plants have been observed with browned downward and backward turned tips or with a slight pinching and browning or chlorosis of the leaf margins ¼ inch to an inch from the tip. These latter symptoms may occur independently or on plants with typical rings. The browned pinched tips are of frequent occurrence in the suckers which have grown after the plants have been cut. This symptom in the suckers often gives them the appearance of having been injured by frost and would not usually be recognized as a symptom of ringspot except that it is rather frequently present on the suckers of ringspot plants before cutting. Large, yellowish, concentric patterns sometimes occur on dark tobacco instead of the necrotic rings. Similar patterns occur in *Del-*

phinium sp., but attempts to infect tobacco with this disease have been unsuccessful.[5a]

The rather frequent appearance of ringspot in plant-beds suggested the possibility of seed infection. Seed gathered from a ringspot plant were sowed in a flat in the greenhouse. Plants were used from this flat in a number of physiological studies and were carefully watched for any symptoms of ringspot. The disease appeared in at least 6 of the plants from this lot of seed. These plants showed the pinching of the tips of the leaves, were dark in color and developed necrotic dots on some leaves. Tobacco inoculated from such plants developed typical ringspot. Ringspot has retained its properties thruout 15 successive transfers (Table 1), but the infectivity is destroyed by drying.

Cucumber mosaic type 1. (Plate XII, Figures 1 and 2.) Two strains of cucumber mosaic type 1 virus, one from a naturally-infected cucumber plant and the other from a naturally-infected Burley plant, both from the Experiment Station Farm, were compared in 9 successive transfers in tobacco (Table 1). No difference was noted between the two. Three other strains of cucumber mosaic type 1, one from a cucumber plant with typical white pickle fruits as described by Doolittle (6), one from muskmelon (*Cucumis melo*), and one from milkweed (*Asclepias syriaca*). all of which came from a market garden near Clyde, Ohio, on August 17, 1927, were compared in several transfers with the Kentucky strains. All appeared identical with the Kentucky cucumber and tobacco strains. Another strain was obtained from tobacco from Minnesota which was like those from Ohio. Cucumber mosaic type 1 was described as "puff" by Valleau and Johnson (32), with the suggestion that it might be cucumber mosaic. This type is readily transferred from tobacco to cucumber, where it produces the symptoms described by Doolittle (6). Tobacco plants affected with this virus have a general chlorotic appearance with occasional dark green puffed areas. The upper leaves are narrower than in normal plants and may be pinched at the tips (Plate XII, Figure 2). Tobacco plants affected with this virus sometimes develop, on rubbed leaves, necrotic, ring-like patterns similar to

[5a] Since this manuscript was prepared, Dr. W. D. Valleau obtained typical coarse etch symptoms in tobacco from virus-diseased *Delphinium sp.*

those described for coarse etch. More often the first sign of infection is a faint mottling of pale green patterns barely visible in the interveinal areas. Signs of infection may appear a week after inoculation, but generally the incubation period is longer than for any of the other viruses herein described. Greenhouse plants may show a few mild symptoms for a time and then appear healthy for several weeks. They may again develop typical symptoms or may remain entirely normal in appearance until maturity. The virus is present in such plants, as infection is obtained when tobacco is inoculated from them. The infectivity of cucumber mosaic type 1 is destroyed by drying the tissue at room temperature.

Cucumber mosaic type 2. (Plate XIII.) This virus was obtained from a naturally-infected Burley plant growing near a vegetable garden (Table 8, Farm 1) on July 28, 1928. The most conspicuous symptom on tobacco is a mottling formed by the development of pale green to yellow tissue along the veins. There is none of the general chlorosis noted in type 1; the leaves are rarely distorted and the plants are only slightly stunted. This type resembles the yellow cucumber mosaic in tobacco, exhibited by Dr. James Johnson at Ames, Iowa, on December 28, 1929. Small, circular, pale green to yellow spots appear on rubbed leaves in 4 to 10 days after inoculation. These spots do not always develop and then the first symptom appears as pale green to yellow tissue spreading out from the veins. The spots on rubbed leaves resemble those produced by white mosaic, but they are never distinct and they do not become necrotic. Cucumber mosaic type 2 has retained its properties thruout eleven transfers in tobacco (Table 1). No infection was obtained from dried tissue (Table 2).

Cucumber mosaic type 3 virus was obtained from Minnesota from tobacco collected by Mr. H. H. Thornberry. This virus was originally mixed with veinbanding and was obtained free of the latter by inoculating cucumber, which is not susceptible to veinbanding. The symptoms produced by this type on tobacco are of the same general character as those produced by type 1, except that they are in all particulars more prominent. Tobacco plants affected with type 3 are distinguished from those affected with type 1, by the drooping, yellowed lower leaves and mal-

TABLE 1

Successive Transfers of Viruses in Turkish Tobacco in the Greenhouse.

Virus type and source.	Date of first transfer.	Date of last transfer.	Consecutive transfers.[6]	Average number of days to first symptoms.
Severe mosaic type 1. Cured tobacco, 1920.	10/22/25	7/17/29	20	8
Mild mosaic type 1. Cured tobacco, 1920.	12/ 1/25	7/17/29	20	9
Severe mosaic type 2. Burley, Fayette county	7/16/27	7/17/29	12	7
Mild mosaic type 2. Burley, Fayette county	7/19/27	7/17/29	12	7
Yellow mosaic. Burley, Fayette county	7/30/27	7/17/29	12	6
White mosaic. Pepper, Exp. Station Farm	8/10/28	7/17/29	6	5
Ring mosaic. Burley, Fayette county	8/10/27	7/17/29	12	9
Etch. Burley, Fayette county	9/16/26	7/17/29	17	7
Etch (Georgia) Thomasville, Ga.	7/16/28	7/17/29	5	6
Etch+. Burley, Exp. Station farm	8/11/27	7/17/29	11	6
Severe etch. Miamisburg, Ohio	8/20/27	7/17/29	15	5
Veinbanding. Burley, Exp. Station farm	7/23/27	7/17/29	13	7
Coarse etch. Burley, Exp. Station farm	8/10/27	7/17/29	14	7
Ringspot. Burley, Exp. Station farm	8/10/27	8/10/29	15	7
Cucumber mosaic type 1. Cucumber, Exp. Sta. farm	9/ 8/27	7/17/29	10	13
Cucumber mosaic type 2. Burley, Fayette county	7/28/27	7/17/29	11	8
Cucumber mosaic type 3. Tobacco, Minnesota	3/21/28	7/17/29	5	8
"Healthy potato" virus. Cobbler foliage, Exp. Station farm	2/ 5/28	7/17/29	7	9
"Healthy potato" virus. Tomato, Hort. greenhouse	6/20/27	2/27/29	11	9
"Healthy potato" virus +veinbanding. Mosaic cobbler	3/ 5/28	7/17/29	7	7
Cucumber mosaic type 3+veinbanding. Tobacco, Minnesota	10/19/27	7/17/29	12	7

[6]The numbers in this column represent consecutive transfers only. A much greater number of transfers have been made with all viruses.

formed bud leaves of the former. The first symptoms are like those of cucumber mosaic type 1, but they appear, on an average, a few days sooner. Cucumber mosaic type 3 has not been observed in Kentucky tobacco fields. The properties have remained constant in 5 transfers in tobacco (Table 1). The infectivity of this virus is destroyed by drying.

"Healthy potato" virus disease, which appears to be the same as Johnson's "ringspot" (14) was obtained first on June 20, 1927, from a tomato plant in a greenhouse where potato indexing was being done. It was not recognized as a potato virus until comparisons were made some time later with a virus obtained from apparently healthy potatoes of the Cobbler variety. Small chlorotic spots rarely larger than $\frac{1}{16}$ inch in diameter, with a fine necrotic border, may develop on rubbed leaves in 4 to 21 days. After the apparance of the local rings, chlorotic rings and line patterns, $\frac{1}{16}$ to almost an inch in diameter, appear in the leaves above those that were rubbed. These patterns resemble those of watered silk or the watermarks on bond paper, as they are seen better by transmitted light. Frequently necrosis occurs in the light-colored areas of these patterns, resulting in rings and line patterns which might be confused with either coarse etch or ringspot. In the absence of local infection, the first symptom to appear is the watered silk pattern. This disease has not been recognized in tobacco under natural conditions in Kentucky. "Healthy potato" virus does not retain its infectivity in dried tissue (Table 2).

"Healthy potato" virus + veinbanding. This mixed virus disease was obtained from a naturally-infected greenhouse-grown Cobbler potato plant on March 5, 1928. The foliage of the potato plant was mottled and the leaf surfaces were rugose. The disease produced by these two viruses was thought to be due to a single virus. Later Valleau observed that the symptoms of veinbanding were apparent on the older leaves and therefore a mixture of the "healthy potato" virus with veinbanding was used to inoculate tobacco, and symptoms identical to those produced by the virus complex from potatoes resulted. This feature of the problem will receive further attention in a paper by Valleau and Johnson. Plants affected by the two viruses are more severely injured than by either of the viruses

alone. In addition to the small rings on rubbed leaves and vein chlorosis in the growing point leaves, necrosis develops along the veins of these leaves, resulting in a crinkled appearance and frequently in the death of the leaves. The disease produced on tobacco seems to be like the "spot necrosis" described by Johnson (14). There was no separation of the viruses in 7 successive transfers and the infectivity of the mixed virus disease is destroyed by drying (Tables 1 and 2).

Cucumber mosaic type 3 + veinbanding. (Plate XIV, Figure 2.) As previously mentioned, these mixed viruses were obtained from tobacco collected in Minnesota by Mr. H. H. Thornberry. This mixture produces symptoms on tobacco of the same general type as are produced by cucumber mosaic type 3. Besides the chlorosis, distortion, and puffing produced by cucumber mosaic type 3, the dark bands of tissue along the veins, typical of veinbanding, are visible. The mixed viruses cause more stunting and distortion and the lower leaves become yellower and droop more than in the case of plants affected by cucumber mosaic type 3 alone. The incubation period and early symptoms are the same as for cucumber mosaic type 3. There was no separation of the viruses in 12 successive transfers in tobacco and no indication of any change in virulence of the disease.

TABLE 2

Inoculations of Turkish Tobacco in the Greenhouse with Extract of Dried Virus Diseased Tobacco.

Virus type.	Age of Dried Tobacco.	No. of Plants Inoculated.	Number Positive	Days to First Symptoms.	Type Transmitted.
Severe mosaic type 1.....	30 months	6	6	4–11	Severe mosaic type 1
Mild mosaic type 1.....	18 months 10 days	1	1	8	Mild mosaic type 1
Severe mosaic type 2.....	9 months 13 days	1	1	8	Severe mosaic type 2
Severe mosaic type 2	9 months 27 days	3	3	4	Severe mosaic type 2
Yellow mosaic	9 months 27 days	4	4	5	Yellow mosaic
White mosaic	1 month 27 days	4	4	4	White mosaic

Table 2—Continued

Virus type.	Age of Dried Tobacco.	No. of Plants Inoculated.	Number Positive	Days to First Symptoms.	Type Transmitted.
Ring	9 months				
mosaic	27 days	4	4	13	Ring mosaic
Ring	16 months				
mosaic	15 days	5	4	6–10–12	Ring mosaic
Etch	9 months				
	11 days	9	0	.....	.....
Etch	11 months				
	4 days	12	0	.....	.....
Etch	16 months	12	0	.....	.....
Etch+	9 months				
	27 days	5	0	.....	.....
Severe	9 months				
etch	27 days	5	0	.....	.....
Veinbanding	9 months				
	27 days	6	0	.....	.....
Veinbanding	17 months				
	8 days	5	0	.....	.....
Coarse	17 months				
etch	8 days	5	0	.....	.....
Ringspot	31 months				
	8 days	5	0	.....	.....
Cucumber					
mosaic	9 months				
type 1	27 days	5	0	.....	.....
Cucumber					
mosaic	17 months				
type 1	7 days	5	0	.....	.....
Cucumber					
mosaic	17 months				
type 1	27 days	5	0	.....	.....
Cucumber					
mosaic	9 months				
type 2	27 days	6	0	.....	.....
Cucumber					
mosaic	17 months				
type 3	27 days	5	0	.....	.....
"Healthy po-	9 months				
tato" virus	27 days	6	0	.....	.....
"Healthy po-	16 months				
tato" virus	7 days	5	0	.....	.....
Checks. Rubbed with extract from dried healthy tissue	.....	6	0	.....	.....

INOCULATIONS OF TURKISH TOBACCO PLANTS WITH EXTRACT FROM AIR-DRIED TISSUE OF VIRUS-DISEASED TOBACCO

Plants of Turkish tobacco affected with virus diseases were saved from other experiments, allowed to dry at room temperature and kept for different lengths of time. Water extracts from this material were used to inoculate Turkish tobacco plants. Table 2 shows that the infectivity of the viruses, except that of the tobacco mosaics, was destroyed by the air-drying to which they were subjected. This property has been used as one basis for distinguishing the tobacco mosaic viruses from the other viruses in this bulletin.

OVERWINTERING OF VIRUSES IN PERENNIAL SOLANACEOUS WEEDS.

The importance of preventing seed-bed infection with tobacco mosaic has been suggested by a number of workers, Clinton (4), Johnson (13) and Chapman (3). Valleau and Johnson (31) showed that high percentages of mosaic infection were not uncommon in fields, the plants for which were pulled from apparently mosaic-free beds by workmen whose hands had become contaminated by handling natural leaf chewing tobacco, whereas if only sterile tobacco was chewed this disease might be nearly completely controlled. It has been observed in the course of the present studies that those virus diseases that do not withstand drying such as ringspot, etch, etch +, coarse etch, veinbanding, and cucumber mosaic, may be quite prevalent in the field (Tables 8 and 9). Perennial solanaceous weeds are numerous in the tobacco fields of Kentucky, especially in the Burley district, and it was thought that they might be important as a means of carrying these and other viruses thru the winter.

Collections of horsenettle (*Solanum carolinense* L), clammy groundcherry (*Physalis heterophylla* Nees) and groundcherry (*Physalis sp.*) were made at random in and near fields of tobacco on the Experiment Station Farm and in the vicinity of Lexington during the years 1926, 1927 and 1928. Inoculations were made to tobacco, the results of which are given in Table 3.

Severe mosaic types 1 and 2, mild mosaic type 1, and ringspot were obtained from horsenettle. It is believed that these results do not represent all the viruses that may affect this

species because there was difficulty in getting infection on tobacco from specimens showing distinct symptoms in the field, and from greenhouse plants inoculated with known viruses, which seemed to be infected. (See Table 14 and discussion following it.)

In the inoculations from groundcherry, severe mosaic type 1, mild mosaic type 1, yellow mosaic, etch, etch +, severe etch, ringspot, and cucumber mosaic type 1 were obtained.

These results indicate that the perennial solanaceous weeds are potential sources of virus diseases and may be important in the overwintering of these and possibly other viruses.

TABLE 3

Inoculations of Turkish Tobacco in the Greenhouse with Juice from Naturally Infected Virus-Diseased Solanaceous Weeds.

Species from which inoculations were made.	Year inoculations were made	No. of inoculations.	Number positive.	Virus types.
Physalis (sps.)	1926	12	5	2 mild mosaic type 1 3 severe mosaic type 1
Physalis (sps.)	1927	29	21	8 mild mosaic type 1 9 severe mosaic type 1 3 etch 1 ringspot
Physalis (sps.)	1928	93	77	7 mild mosaic type 1 48 severe mosaic type 1 7 severe mosaic type 2 5 yellow mosaic 1 severe etch 1 etch 5 etch+ 3 cucumber mosaic type 1
Physalis heterophylla..	1927	8	5	2 severe mosaic type 1 2 mild mosaic type 1 1 etch with severe mosaic type 1
	1928	5	4	1 severe mosaic type 1 2 etch 1 etch+
Solanum carolinense....	1926	6	3	3 severe mosaic type 1
	1927	58	20	10 severe mosaic type 1 9 mild mosaic type 1 1 ringspot
	1928	101	35	16 severe mosaic type 1 15 mild mosaic type 1 4 severe mosaic type 2

INOCULATIONS FROM NATURALLY INFECTED CULTIVATED SOLANACEOUS AND CUCURBITACEOUS PLANTS TO TURKISH TOBACCO

Frequent reference is made in the literature to the mosaic diseases of the tomato (*Lycopersicon esculentum*), pepper (*Capsicum frutescens*), cucumber (*Cucumis sativus*) and petunia. Some of the descriptions of affected tomato, pepper, and petunia are not entirely typical of the symptoms of tobacco mosaics on these plants. It was therefore thought advisable to inoculate tobacco from infected plants of these species and of muskmelon (*Cucumis melo*), in order to determine whether the infections were of more than one type. The results of these inoculations are given in Tables 4, 5 and 6.

At least 6 virus diseases were obtained from naturally-infected pepper plants: severe mosaic type 1, white mosaic,

TABLE 4

Inoculations of Turkish Tobacco in the Greenhouse with Juice from Naturally Infected Virus-Diseased Peppers.

Source.	Date of Inoculation.	Days to first symptoms.	Virus type.
Fayette County, Ky.	9/13/26	6	Severe mosaic type 1 mixed with etch
Fayette County, Ky.	9/28/26	6	Severe mosaic type 1
Fayette County, Ky.	6/18/28	17	Cucumber mosaic type 1
Fayette County, Ky.	6/18/28	17	Cucumber mosaic type 1
Kentucky Experiment Station farm	8/ 6/28	8	Severe etch
Kentucky Experiment Station farm	8/ 6/28	24	Severe etch
Kentucky Experiment Station farm	8/ 6/28	5	Etch+
Kentucky Experiment Station farm	8/ 6/28	8	Etch+
Kentucky Experiment Station farm	8/ 6/28	8	Etch+
Kentucky Experiment Station farm	8/ 6/28	8	Etch
Kentucky Experiment Station farm	8/ 6/28	10	Etch
Kentucky Experiment Station farm	8/ 6/28	5	Severe mosaic type 1
Kentucky Experiment Station farm	8/10/28	5	White mosaic

etch, etch +, severe etch, and cucumber mosaic type 1. This probably does not represent the viruses that may be expected because a total of only 13 inoculations was made (Table 4). The plants from which these inoculations were made were growing in market gardens.

Eight virus diseases: severe mosaic type 1, mild mosaic type 1, etch, etch +, severe etch, veinbanding, coarse etch, and "healthy potato" virus were obtained in 28 inoculations from naturally-infected tomatoes.

The inoculations from naturally-infected cucumber and muskmelon resulted in ringspot and cucumber mosaic type 1. Cucumber mosaic type 1 seems to be of rather frequent occur-

TABLE 5

Inoculations of Turkish Tobacco in the Greenhouse with Juice from Naturally Infected, Virus-Diseased Tomato.

Source.	Date of Inoculation.	Days to first symptoms.	Virus type.
Fayette County, Ky.	9/13/26	13	"Healthy potato" virus+ severe mosaic type 1
Fayette County, Ky.	9/28/26	9	Mild mosaic type 1
Fayette County, Ky.	9/28/26	7	Etch
Fayette County, Ky.	9/28/26	7	Severe mosaic type 1
Fayette County, Ky.	9/28/26	7	Severe mosaic type 1
Fayette County, Ky.	9/28/26	7	Severe mosaic type 1
Fayette County, Ky.	9/28/26	7	Severe mosaic type 1
Fayette County, Ky.	9/28/26	7	Severe mosaic type 1 mixed with etch
Fayette County, Ky.	6/18/28	8	Veinbanding
Fayette County, Ky.	6/18/28	8	Veinbanding
Fayette County, Ky.	6/18/28	8	Veinbanding
Horticultural greenhouse	6/20/27	8	"Healthy potato" virus
Horticultural grounds	6/20/27	8	Severe mosaic type 1
Garden in rear of Experiment Station greenhouse	6/19/28	4	Etch+
Garden in rear of Experiment Station greenhouse	6/19/28	6	Severe etch
Garden in rear of Experiment Station greenhouse	6/19/28	7	Coarse etch (?)
Garden in rear of Experiment Station greenhouse	6/26/28	5	Etch+(12 samples, 12 positive)

TABLE 6

Inoculation of Turkish Tobacco in the Greenhouse with Juice from Naturally Infected, Virus-Diseased Cucumber, Muskmelon and Petunia.

Species.	Source.	Date of Inoculation.	Days to first symptoms.	Virus type.	Remarks.
Cucumber	Tobacco field, Fayette County, Ky.	8/10/27	13	Ringspot	Tobacco field had 5.7% ringspot in the tobacco.
Cucumber	Clyde, Ohio. (5 specimens)	8/20/27	18	Cucumber mosaic, type 1	Field had a large number of mosaic infected milkweed.[7]
Cucumber	Fayette County, Ky.	9/ 7/27	17	Cucumber mosaic, type 1	
Cucumber	Hardinsburg, Ky. (4 specimens)	7/ 3/28	7	Ringspot	
Muskmelon	Ky. Exp. Sta. farm.....	8/13/28	7	Cucumber mosaic type 1	
Muskmelon	Ky. Exp. Sta. farm.....	8/13/28	11	Ringspot	
Petunia	Ky. Exp. Sta. farm.....	6/27/28	6	Severe etch	
Petunia	Ky. Exp. Sta. farm.....	7/17/28	21	"Healthy potato" virus+ veinbanding	
Petunia	Ky. Exp. Sta. farm.....	7/17/28	6	Cucumber mosaic type 1	Greenhouse garden.[8]

Petunia	Ky. Exp. Sta. farm.....	7/17/28	6	Cucumber mosaic type 1
Petunia	Ky. Exp. Sta. farm.....	7/17/28	12	Cucumber mosaic type 1
Petunia	Ky. Exp. Sta. farm.....	7/17/28	12	Coarse etch
Petunia	Ky. Exp. Sta. farm.....	7/17/28	12	Severe etch
Petunia	Ky. Exp. Sta. farm.....	7/17/28	6	Etch+
Petunia	Ky. Exp. Sta. farm.....	8/10/28	10	Coarse etch
Petunia	Ky. Exp. Sta. farm.....	8/10/28	20	Coarse etch
Petunia	Ky. Exp. Sta. farm.....	8/10/28	14	Cucumber mosaic type 1
Petunia	Ky. Exp. Sta. farm.....	8/10/28	10	Mild mosaic type 1
Petunia	Ky. Exp. Sta. farm.....	8/10/28	10	Etch
Petunia	Ky. Exp. Sta. farm.....	8/10/28	10	Severe etch
Petunia	Ky. Exp. Sta. farm.....	8/13/28	23	Cucumber mosaic type 1
Petunia	Ky. Exp. Sta. farm.....	8/13/28	23	Cucumber mosaic type 1

[7]Inoculations to tobacco from two infected milkweed plants (*Asclepias syriaca L*) from this field gave cucumber mosaic type 1.

[8]The next 13 inoculations were from plants set in the rear of the greenhouse in which these studies were made. The viruses obtained probably represent insect spread, as the plants were not handled thruout the growing season.

rence in cucumber in Kentucky, and ringspot is not uncommon. Muskmelon seems to be less often affected with either of these virus diseases.

From *Petunia sp.*, 8 virus diseases were obtained, namely: mild mosaic type 1, etch, etch +, severe etch, coarse etch, cucumber mosaic type 1, veinbanding, and "healthy potato" virus. Most of these inoculations were from plants set in the rear of the greenhouse in which these studies were made, and near where infected tobacco plants were discarded on a heap, which gave ample opportunity for insect transmission to the petunias. It may be that all these types do not occur in petunias in the field, but it is an indication of what might be expected if sources of inoculum are present.

VIRUS DISEASES IN NATURALLY INFECTED TOBACCO

In the summer of 1927 inoculations were made to Turkish tobacco in the greenhouse from tobacco plants naturally infected with virus diseases in the field to discover how many of the virus diseases described in this bulletin and by other workers were present. These inoculations were made from plants which showed symptoms of virus diseases readily recognized by the writer as typical for known virus diseases and also from plants with atypical symptoms. The inoculations were started June 21, 1927, about 3 weeks after setting, and continued until September 30, 1927. During this time the cigar tobacco district of Ohio was visited and inoculations were made from tobacco samples collected there. Inoculations were also made from virus-diseased tobacco sent from Minnesota and Georgia. The results of the inoculations are given in Table 7. The data do not show the distribution or prevalence of the different types. These are given in Tables 8 and 9 for fields near Lexington.

Of the virus diseases described in this bulletin and listed in Table 1, all except white mosaic, etch (Georgia), cucumber mosaic type 3, and the "healthy potato" virus were found in Burley tobacco. Ring mosaic and severe etch are of infrequent occurrence, while the others are found without difficulty in some areas. Ringspot, etch, severe etch, severe mosaic type 1, mild mosaic type 1, and cucumber mosaic type 1 were obtained from the samples collected in Ohio. Cucumber mosaic types 1

and 3 and veinbanding were obtained from the Minnesota collection. The inoculation from the Georgia tobacco gave only etch. The inoculations indicate the types which may be found in some fields of tobacco in Kentucky and elsewhere. More extensive inoculations might reveal types not herein described.

TABLE 7

Inoculations of Turkish Tobacco in the Greenhouse with Juice from Naturally Infected, Virus-Diseased Tobacco.

Source of infected plant.	Tobacco type.	No. of Inoculations.	Virus types transmitted.
Kentucky Experiment Sta. farm	Burley	73	4 severe mosaic type 1
			2 severe mosaic type 2
			4 mild mosaic type 1
			1 yellow mosaic
			1 ring mosaic
			16 etch
			2 etch+
			1 severe etch
			8 veinbanding
			10 ringspot
			11 cucumber mosaic type 1
			5 coarse etch
Fayette County, Kentucky	Burley	21	1 severe mosaic type 1
			1 severe mosaic type 2
			1 mild mosaic type 1
			1 mild mosaic type 2
			5 etch
			1 etch+
			4 veinbanding
			1 ringspot
			5 cucumber mosaic type 1
			1 cucumber mosaic type 2
Miamisburg, Ohio	Cigar	10	1 severe mosaic type 1
			1 mild mosaic type 1
			1 etch
			2 severe etch
			1 ringspot
			4 cucumber mosaic type 1
Minnesota	Cigar	8	1 veinbanding
			1 cucumber mosaic type 1
			1 cucumber mosaic type 3
Georgia	Dark Tobacco	7	6 etch (Georgia type)

OBSERVATIONS ON THE OCCURRENCE AND SEASONAL DISTRIBUTION OF VIRUSES IN BURLEY TOBACCO

The various plots of tobacco on the Experiment Station Farm were inspected from 2 to 4 times a season for virus diseases and records made during the years 1927, 1928 and 1929. The first observation was made 2 or 3 weeks after setting the tobacco and subsequently at intervals of 2 to 4 weeks. The purpose was to obtain information on the prevalence and seasonal distribution of virus diseases in tobacco on the Experiment Station farm. A record of these observations is given in Table 8.

It has been found that if tobacco plants are pulled with clean hands, the percentage of mosaic infection is very low as late as a month after setting. Two weeks after setting, when the first observations are made, the number of virus diseases is almost negligible. Two or three weeks later, etch etch +, ringspot, and occasionally coarse etch, cucumber mosaic, and veinbanding appear. There is a gradual increase in numbers of plants affected with tobacco mosaics, etch, etch +, ringspot, coarse etch, cucumber mosaic, and veinbanding as the season advances. The apparent decrease in numbers of etch, etch +, cucumber mosaic, and coarse etch in late observations is believed to be due to the inability to recognize these diseases in tobacco that is turning yellow because of maturity. The fourth observation was made after topping and suckering, and relatively high percentages of tobacco mosaics at this time are possibly due to mechanical spread by these operations. Veinbanding is always present in fields on the Experiment Station farm at the time of the fourth observation. It is not known whether the virus of veinbanding is spread readily by hand, but observations indicate that in some areas it may be present in a large percentage of plants before any of the operations necessary in caring for the crop have been performed (Table 9, farms 17, 18, 19, 20, 21 and 22a). In the field, the veinbanding symptom (Plate X, Figure 1) is used as a means of recognizing this disease and, as this symptom develops slowly in some plants, there is a possibility that the plants may have been infected for some time and that the higher percentages late in the season may be more apparent than real.

TABLE 8

Occurrence and Seasonal Distribution of Tobacco Viruses in Burley Tobacco on the Experiment Station Farm, Lexington, Ky. Average Percentages.

Tobacco	Tobacco Mosaic %	Etch %	Etch + %	Ringspot %	Coarse etch %	Cucumber mosaic %	Veinbanding %
Field A. Averages of 1927, 1928 and 1929.							
1st inspection. (14,410 plants) ..	.09	0	0	.005	0	0	.01
2nd inspection. (14,392 plants) ..	.27	.28	.13	.41	.05	.125	.01
3rd inspection. (14,342 plants) ..	.59	1.73	.33	.93	.17	.50	.87
4th inspection. (11,528 plants) ..	5.25	2.05	.80	1.22	.12	.70	4.81
Field B. Averages of 1927, 1928 and 1929.							
1st inspection. (24,349 plants) ..	.022	.007	0	.021	0	0	0
2nd inspection. (24,349 plants) ..	.09	.13	.01	.45	.02	.09	0
3rd inspection. (24,317 plants) ..	.29	.25	.10	1.61	.08	.35	.50
4th inspection. (24,310 plants) ..	1.11	.12	.08	1.37	.05	.30	1.04
Field C. Averages of 1927, 1928 and 1929.							
1st inspection. (2,982 plants)	.52	0	0	.02	0	0	0
2nd inspection. (2,978 plants)	.74	.28	0	1.36	.03	.02	.18
3rd inspection. (2,978 plants)	1.42	.91	.06	3.10	.18	.09	.62
Field D. Averages of 1928 and 1929.							
1st inspection. (3,478 plants)	0	0	0	.01	0	0	0
2nd inspection. (3,478 plants)	.04	.09	.01	.75	.01	.07	0
3rd inspection. (3,478 plants)	.25	.15	.04	4.78	.15	.17	.65
4th inspection. (3,478 plants)	.35	.07	.25	6.36	.03	.25	1.22
Field E. Averages of 1928 and 1929.							
1st inspection. (2,645 plants)	.02	0	0	.01	0	0	0
2nd inspection. (2,645 plants)	.13	.03	.03	.33	.04	.06	0
3rd inspection. (2,645 plants)	.70	.66	.07	1.62	.10	.10	.23
4th inspection. (2,645 plants)	1.99	.15	.12	2.05	.01	.11	1.10

Table 8—Continued

Tobacco	Tobacco Mosaic %	Etch %	Etch + %	Ringspot %	Coarse etch %	Cucumber mosaic +	Veinbanding %
Field F. Averages of 1928 and 1929.							
1st inspection. (2,653 plants)	.02	.02	0	0	0	0	0
2nd inspection. (2,653 plants)	.22	.34	.07	.31	0	0	0
3rd inspection. (2,653 plants)	.97	1.08	.16	1.68	.06	.05	.26
4th inspection. (2,653 plants)	3.56	.20	.72	2.05	.04	.09	1.05
Field G. Averages of 1927, 1928 and 1929.							
1st inspection. (2,868 plants)	.22	0	.01	.06	.01	0	0
2nd inspection. (2,815 plants)	.29	1.75	.52	1.05	.30	.22	.33
3rd inspection. (2,797 plants)	1.88	.20	.07	1.70	.09	.14	1.23
Field H. Averages of 1927, 1928 and 1929.							
1st inspection. (2,008 plants)	.02	.06	.01	.18	0	0	0
2nd inspection. (2,000 plants)	.26	.96	.57	1.40	.31	.17	.41
Field I. Averages of 1927, 1928 and 1929.							
1st inspection. (1,708 plants)	.15	.13	0	0	0	0	0
2nd inspection. (1,708 plants)	.91	2.00	.51	.40	.06	.12	.69
3rd inspection. (1,708 plants)	2.97	3.16	7.75	1.62	.04	.40	11.22
Field J.[9] Averages of 1927 and 1928.							
1st inspection. (9,873 plants)	1.18	.10	.05	.43	0	0	0
2nd inspection. (5,600 plants)	4.94	12.59	.97	6.84	.31	.50	3.83
Field K.[9] 1929.							
1st inspection. (2,854 plants)	1.79	0	0	0	0	0	0
2nd inspection. (2,854 plants)	4.39	.35	.38	.95	.11	.46	.60
3rd inspection. (2,854 plants)	7.43	.28	1.23	1.58	.25	.92	3.22

[9]No precautions taken to prevent infection at pulling time with tobacco mosaics in fields J and K.

The importance of solanaceous weeds as potential carriers of the virus diseases should be noted again. These are abundant in and around all the fields on the Experiment Station farm and many of them are infected (Table 3).

The importance of preventing infection with tobacco mosaics while pulling the plants is shown in fields J and K, where no precautions were taken by the workmen who pulled the plants. The initial infections from tobacco mosaics are strikingly higher in these fields than in any others listed in the table.

Inspections were made also in fields of tobacco near Lexington during the same years in which those above were made. These are recorded in Table 9. It is to be observed that tobacco mosaics occur in greater numbers on these farms than on the Experiment Station farm, where precautions were taken to prevent infection while handling the plants at setting time. It is also interesting to note that the percentage of etch, cucumber mosaic, and veinbanding are high on those farms in vegetable and potato-growing areas (Farms 1, 13, 15, 18, 19, 20, 21 and 22a).

TRANSMISSIBILITY STUDIES

Transmissibility studies were made, with most of the viruses, in the field and greenhouse both for the purpose of obtaining some knowledge of their symptoms on different hosts and to ascertain whether any particular virus might be modified by its sojourn in hosts other than tobacco. Most transfers were made to Turkish tobacco from the various species tested. If symptoms resulted on tobacco and were characteristic of the type with which the species was inoculated, it was assumed that there had been no change in the virus and that the species was susceptible to it.

GREENHOUSE INOCULATIONS

To tomato. The results of inoculations of tomato (*Lycopersicon esculentum*) with the viruses are given in Table 10. Two plants of the variety June Pink were grown in forest soil in half-gallon jars and were kept in a vigorously growing condition by bi-weekly applications of nitrate. The inoculations were made when the plants were six inches tall. Positive results were obtained with all the viruses except ringspot and coarse etch.

TABLE 9.

Occurrence of Virus Diseases in Burley Tobacco on Farms in Fayette County, Ky.[10]

Farm No.	Date of Inspection.	Number of plants.	Tobacco mosaic %	Etch %	Etch + %	Ringspot %	Coarse etch %	Cucumber mosaic %	Veinbanding %	Remarks
1	7/12/27	3,810	.03	0	0	0	0	0	0	Vegetable gardener.
2	7/14/27	2,147	.93	1.30	0	.65	0	0	0	Grower did not use tobacco.
3	7/14/27	3,789	7.20	.13	0	.72	0	0	0	Grower chewed natural leaf tobacco.
4	7/14/27	5,929	1.88	0	0	.22	0	0	0	
5	7/16/27	7,000	.90	0	0	5.70	0	0	0	Several ringspot plants found in bed.
6 (a)	7/14/27	7,620	.90	0	0	.30	0	0	0	(6a, b, c, d, 4 fields on one farm in 4 different areas. Beds handled by tenants and hired labor, all of whom chewed tobacco while handling plants.)
(b)	7/16/27	4,244	1.80	0	0	.40	0	0	0	
(c)	7/16/27	1,842	6.70	0	0	.90	0	0	0	
(d)	7/16/27	1,416	3.80	0	0	.50	0	0	0	
7	7/18/27	5,145	2.14	0	0	.16	0	0	0	
8	7/19/27	8,226	4.70	.54	0	.06	0	0	0	
9	7/19/27	2,218	.40	2.79	0	0	0	0	0	
10	7/29/27	6,400	24.49	0	0	0	0	0	0	
11	7/29/27	1,000	2.30	0	0	9.50	0	0	0	
12 (a)	7/29/27	2,080	5.91	1.16	0	.32	0	0	.14	12(a) and (b) 2 fields on same farm.
(b)	7/29/27	1,166	11.40	0	0	.51	0	0	.17	
1	7/29/27	10,608	.008	.42	0	.21	0	.21	2.79	Later observation on farm 1.

13		7/29/27	2,060	.97	.15	0	.45	0	0	1.06	Near farm 1. Near potato field.
14		7/29/27	3,864	.59	.41	0	.15	0	0	.05	Grows potatoes for market.
15		7/17/28	2,872	.87	.12	0	.42	0	.03	0	Plants from same bed as those used in field J, table 8. Planted by same men 3 miles from J.
16		7/17/28	1,272	1.02	.94	0	.31	.07	1.88	.47	Garden plot not plowed for 10 years. Plants from same bed as those in 15.
17		7/24/28	966	3.51	1.34	0	.62	.10	1.24	3.41	Garden plot. Plants from same bed as 15 and 16.
18		7/13/29	999	7.10	26.50	0	1.70	.50	1.20	2.20	Vegetable garden area.
19		7/15/29	2,000	14.25	13.75	.10	1.60	0	.55	7.50	Vegetable garden area.
20		7/15/29	2,350	4.89	12.12	.08	.72	0	1.23	37.91	Vegetable garden area.
21		7/16/29	1,082	8.32	33.36	0	1.29	.09	1.20	5.17	Across highway from 19.
22	(a)	7/20/29	412	0	23.81	0	1.94	0	0	7.04	(a) Small plot formerly used as a garden. Grower used no tobacco while handling plants.
	(b)	7/20/29	1,270	5.82	1.81	0	.39	0	0	.08	(b) Large field set by tenant just over fence from (a). Tenant used tobacco while pulling plants.
23	(a)	6/26/29	3,910	.12	0	0	.07	0	0	0	Sanitary precautions used in pulling plants.
	(b)	8/16/29	4,400	1.29	.06	.04	.59	0	0	.11	Later count in 23.

[10]The inspections were made previous to topping and suckering of the crop.

The four mosaics, mild, types 1 and 2, and severe, types 1 and 2, produce symptoms that differ from each other in degree only. All these cause mottling characterized by dark green, irregular areas associated with the veins. The mild mosaics cause little or no distortion, and the mottling is obscure, often being visible only in vigorously growing plants. Mottling is readily visible in plants affected with the severe mosaics; both cause distortion consisting of narrowing and twisting of the young leaflets, and both cause some general chlorosis and stunting (Plate XV, Figure 2). The differences between the severe types are observable only by direct comparison of infected plants.

Yellow mosaic causes the same type of distortion, mottling and stunting as the severe mosaics but, in addition, the areas between the veins are distinctly lemon-yellow, and yellow, irregular blotches of different sizes up to ¼ inch in diameter appear on leaflets that show no other symptom.

Ring mosaic virus produces very striking symptoms on tomato. Circular, water-soaked necrotic spots, ¼ inch in diameter, develop on rubbed leaves in 7 to 10 days after inoculation. Necrotic streaks ⅛ to ½ inch long develop on the midveins of these leaves a few days later. Leaflets affected in this way (Plate XVI, Figure 1) usually droop markedly. The necrosis gradually spreads from the midvein to the lamina and eventually the leaflet dies. Streaks gradually form in the main axis of the leaf and then in the stem (Plate XX, Figure 9). Narrow yellow areas develop along the veins of unrubbed leaflets and these develop the sequence of symptoms described above. Yellowed areas may occur as oakleaf-like patterns, and concentric yellow patterns as large as ¼ inch in diameter have been observed between the veins. Concentric, sunken, chlorotic rings, ⅛ to ¾ inch in diameter, appear on the fruit when the latter is about half grown (Plate XVI, Figure 3). These areas on the fruit may become necrotic. The streak symptoms resemble those produced by a combination of tobacco mosaic and a potato virus as described by Vanterpool (34), and Stover (27). Tomato plants were inoculated with a mixture of severe mosaic type 1 and "healthy potato" virus for comparison with plants affected with ring mosaic. This combination produced streak of the midrib and stem and necrosis of the leaves, but yellow rings

TABLE 10

Inoculations of Tomato in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive	Days to first symptoms	Inoculations back to Turkish tobacco			
				No. plants inoculated	Number positive	Days to first symptoms	Type transmitted.
Severe mosaic type 1	3	3	16–18	3	3	5	Severe mosaic type 1
Mild mosaic type 1	1[11]	1	18	3	3	6	Mild mosaic type 1
Mild mosaic type 1	1	1	7	1	1	10	Mild mosaic type 1
Severe mosaic type 2	3	3	18	3	3	5	Severe mosaic type 2
Mild mosaic type 2	1	1	18	3	3	5	Mild mosaic type 2
Yellow mosaic	2	2	18	3	3	5–6	Yellow mosaic
Ring mosaic	2	2	19–25	3	3	16	Ring mosaic
				6	6	11	
	2	2	8	0	....	.....	.....
	10[12]	10	18	0	....	.....	.....
	2	2	10–12	2	2	7	Ring mosaic
Etch	2	2	22	3	3	5–6	Etch
	2	2	12	2	2	7	Etch
Etch+	2	2	18	3	3	5–9	Etch+
	1	1	9	2	2	7	Etch+
Severe etch	2	2	7	0	....	.....	.....
	2	2	7	2	2	7	Severe etch
Veinbanding	2	2	15	3	3	13–15	Veinbanding
Coarse etch	2	0	.....	3	0	.....	.....
	1	0	.....	0	....	.....	.....
	2	0	.....	2	0	.....	.....
Ringspot	2	0	.....	3	0	.....	.....
	2	0	.....	0	....	.....	.....
	2	0	.....	2	0	.....	.....
Cucumber mosaic type 1	2	1	17	3	3	9	Cucumber mosaic type 1
	4	4	8	4	4	7–9	Cucumber mosaic type 1

[11]The inoculations were made in January, March and December. The numbers under each type in this column represent inoculations at different times. The transfers back to turkish tobacco were made after 3 to 5 weeks.

[12]From material dried at room temperature for 65 days.

Table 10—Continued

Virus type	Number plants inoculated.	Number positive	Days to first symptoms	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Cucumber mosaic type 2	1	1	13	2	0	.....	.....
"Healthy potato" virus	2	2	8	0	....	.....	.....
	1	1	15	0	....	.....	.....
	4	4	13	2	2	9	"Healthy potato" virus
	4	4	11	4	4	5–17	"Healthy potato" virus
Cucumber mosaic type 3+veinbanding	2	2	16	3	3	5–7	Cucumber mosaic type 3 +veinbanding
	1	1	20	2	2	8	
Checks	4	0	.....	2	0	.....	.....
Checks. Rubbed with juice from healthy tobacco	2	0	.....	2	0	.....	.....

were not observed on the leaves nor were rings produced on the fruit. Ten tomato plants were inoculated with extract from a ring mosaic tobacco plant that had been air-dried 65 days, and streak symptoms typical of ring mosaic developed in all. "Healthy potato" virus does not survive drying at room temperature, therefore it is believed that ring mosaic is a true tobacco mosaic that produces a streak disease on tomato similar to that described by Vanterpool (34).

The symptoms produced by etch are not striking and affected plants might, on casual inspection, be considered healthy. This virus causes a slight hooking back of the tips of the leaflets together with the inrolling of their margins (Plate XVII, Figure 1). The tissue between the veins is of a paler green color and the lower leaves have a chlorotic cast not present in healthy controls. The leaves are smaller than in healthy plants (compare Plate XIX, Figure 2, with Plate XX, Figure 7).

The leaflets at the growing points of plants affected with etch + are narrow, usually less than half the width of healthy leaflets, and are twisted in such a way as to form spirals (Plate

XVII, Figure 2). The leaves are much reduced in size and the individual leaflets are distorted (Plate XX, Figure 1). Mottling consists of irregular areas of chlorotic tissue between, and sometimes along, the veins and might be confused with the mottling produced by the tobacco mosaics.

Severe etch produces symptoms of the same general type as etch +, but in more conspicuous form. The leaflets are narrow and distorted and have a wrinkled surface. The lower leaves develop chlorosis at the tips of the leaflets. These areas gradually become necrotic and the leaves die. Mottling is more prominent than in plants affected with etch +.

The veinbanding virus, tho easily transferred to tobacco from affected plants, produces very mild symptoms on tomato. In comparison with healthy controls, affected plants have veins that are pale green and a barely visible spotting consisting of irregular to circular pale green areas occurs between the veins of leaves near the growing point. Affected plants are not stunted and without healthy controls for comparison, it is difficult to recognize diseased plants.

Cucumber mosaic type 1 is not always readily transferred to tomato by the method employed. This type produces more striking symptoms on tomato than any of the tobacco mosaics except ring mosaic. Leaves at the growing points have leaflets that are "fern leaf"-like, with margins almost to completely entire. The other leaves are reduced in size and distorted (Plate XX, Figure 3). Affected plants are very much dwarfed (Plate XV, Figure 3) and have a general chlorotic appearance. Some leaves have a mosaic-like mottling and a roughened surface due to the development of pimple-like areas between the veins.

Cucumber mosaic type 2 produces symptoms on tomato that are distinct from those produced by cucumber mosaic type 1. Two weeks after inoculation large, pale green areas appear on rubbed leaves and these areas soon become necrotic. Concentric, ring-like areas of pale green and yellow tissue, ¼ inch in diameter, develop between the larger veins of the unrubbed leaves, and pale green areas develop along the veins. No stunting or distortion was observed in the affected plants.

Cucumber mosaic type 3 + veinbanding causes a marked

stunting and distortion of the tomato (Plate XVII, Figure 3). The individual leaflets at the growing points are very narrow and their margins are almost to completely entire. Some of the leaves at the growing point have a shortened midrib and in some plants, after two almost normal leaflets form, the others develop in a string-like manner (Plate XX, Figure 2). In other leaves, no normal leaflets are formed (Plate XX, Figure 5). This shortening of the main axis of the leaf, together with the abnormal development of the leaflets, gives the plants a "bunchy" or rosette appearance (Plate XVII, Figure 3).

"Healthy potato" virus causes a faint mottling on tomato which consists of pale green tissue irregularly distributed along the veins. About two weeks after inoculation and usually after the appearance of mottling, circular, pale green to almost yellow areas develop on rubbed leaves. This virus causes neither necrosis nor stunting in the greenhouse.

Attempts to transfer ringspot and coarse etch to tomato by the rubbing method were unsuccessful.

Inoculations were made from the unrubbed leaves of all tomato plants to tobacco, and positive results were obtained from all plants except the ones inoculated with ringspot, coarse etch, and cucumber mosaic type 2 (Table 10).

To pepper. The results of inoculating bell pepper are given in Table 11. Severe mosaics, types 1 and 2, produce small chlorotic spots on rubbed leaves in 5 to 12 days. A mosaic-like mottling is visible either at the time the local spots appear or a few days later. Plants affected with these viruses are only slightly distorted in the greenhouse.

Mild mosaics, types 1 and 2, cause spotting and mottling but no distortion.

Yellow mosaic causes the same symptoms as the severe mosaics but, in addition, yellow areas occur between the veins, often only on the basal half of the leaf.

Plants affected with ring mosaic have the local spotting but the mottling is obscure. The rubbed leaves eventually turn yellow. Ring-like patterns composed of alternate bands of green and yellow tissue appear on rubbed leaves after variable periods. Pepper plants affected with ring mosaic are severely distorted and dwarfed.

TABLE 11

Inoculations of Pepper in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive	Days to first symptoms	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic	1[13]	1	12	0	....	.....	Severe mosaic
type 1	2	2	5	1	1	5	type 1
Mild mosaic	1	1	12	0	....	.....	Mild mosaic
type 1	2	2	5	1	1	5	type 1
Severe mosaic	1	0	.....	0	....	.....	Severe mosaic
type 2	2	2	5	1	1	5	type 2
Mild mosaic	1	1	12	0	....	.....	Mild mosaic
type 2	2	2	5	1	1	5	type 2
Yellow	1	1	10	0	....	.....	
mosaic	2	2	7	2[14]	2	5	Yellow mosaic
White mosaic	2	2	9	2[14]	2	3	White mosaic
Ring	1	1	12	0	....	.....	.....
mosaic	2	2	7	2	2	6	Ring mosaic
Etch	1	0	.....	0	....	.....	.....
	2	2	11	2	2	5	Etch
Etch (Ga.)	2	1	7	2	0	.....	.....
Etch+	1	1	11	0	....	.....	.....
	2	1	11	2	2	5	Etch+
Severe	1	1	15	0	....	.....	.....
etch	2	2	11	2	1	5	Severe etch
Veinbanding	1	0	.....	0	....	.....	.....
	2	3	?	2	2	18	Veinbanding
Coarse	1	0	.....	0	....	.....	.....
etch	2	0	.....	2	0	.....	.....
Ringspot	2	0	.....	0	....	.....	.....
	2	0	.....	2	0	.....	.....
Cucumber mosaic	2	0	.....	0	....	.....	.....
type 1	4	2	11	4	0	.....	.....
Cucumber mosaic	1	0	.....	0	....	.....	.....
type 2	2	2	11	2	0	.....	.....
Cucumber mosaic							Cucumber mosaic
type 3	2	2	11	2	1	11	type 3
"Healthy po-	2	2	20	0	....	.....	.....
tato" virus	3	2	7	2	0	.....	.....
"Healthy potato"							
virus+vein-	1	1	20	0	....	.....	"Healthy po-
banding	2	2	7	2	1	23	tato" virus

[13]The numbers under each type in this column represent inoculations at different times. The first inoculation was made on March 9, 1928, and the second on June 10, 1929. The inoculations back to Turkish tobacco were made on July 15, 1929.

[14]Yellow and white mosaic killed the pepper plants. The inoculations to Turkish tobacco are from dead pepper plants.

Table 11—Continued

Virus type	Number plants inoculated.	Number positive	Days to first symptoms	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Cucumber mosaic type 3+vein-banding	2	2	7	2	1	8	Cucumber mosaic type 3+vein-banding

White mosaic produces symptoms not unlike those caused by the severe mosaics but the leaves turn yellow and begin to die soon after the first symptoms appear. Three weeks after the first symptoms appear, the lower leaves are dead and the new leaves are distorted and have pale-yellow to almost white tissue between the veins. In one series of inoculations the plants were killed by this mosaic.

Etch, etch +, and severe etch produce a mottling on pepper that is not readily distinguishable from the mottling that occurs with the tobacco mosaics. Local infections have not been observed with these types, and the plants are seldom noticeably distorted. There is some dwarfing and fewer fruits are produced.

Veinbanding produces small, faintly visible chlorotic spots between the veins. This symptom is not distinct and it is difficult to distinguish diseased plants from healthy ones.

All the cucumber mosaics and cucumber mosaic type 3 + veinbanding produce the same general type of symptoms. Small, concentric, pale green to almost yellow rings appear on rubbed and unrubbed leaves in 7 to 11 days after inoculation. Concentric, ring-like, chlorotic patterns develop along the veins of the lower leaves. With cucumber mosaic type 3 and cucumber mosaic type 3 + veinbanding the concentric rings are larger, sometimes ½ inch in diameter, and the plants are distorted.

"Healthy potato" virus and "healthy potato" virus + veinbanding produce chlorotic rings ⅛ inch in diameter on

rubbed leaves in 7 to 21 days after inoculation. In addition to the local symptoms, the newer leaves have a mottling produced by the development of pale green tissues spreading out from the veins. Necrosis, followed by defoliation, sometimes occurs with the mixed virus disease but this has never occurred with the "healthy potato" virus alone.

The inoculations with the ringspot and coarse etch viruses appeared negative.

Transfers were made from the unrubbed leaves of affected plants to tobacco and infection resulted from all plants except those inoculated with etch (Georgia), cucumber mosaic types 1 and 2, and "healthy potato" virus. "Healthy potato" virus symptoms resulted from the pepper plant inoculated with "healthy potato" virus + veinbanding.

To groundcherry. (*Physalis heterophylla* Nees var. *nyctaginea* (Dunal) Rydb.) The results of the inoculations to this perennial species of groundcherry are given in Table 12. The symptoms produced by 7 mosaics are of the same general type. Chlorotic spots ⅛ to ¼ inch in diameter develop on rubbed leaves about 7 days after inoculation. These spots are not conspicuous in plants affected with the severe and mild mosaics, and only rarely does a spot appear on unrubbed leaves. After 3 weeks these plants appear almost normal and are not stunted. Yellow, white, and ring mosaics produce these spots in greater abundance on both rubbed and unrubbed leaves. The spots are more conspicuous, being of a paler color and larger, sometimes becoming ¾ of an inch in diameter. None of the mosaics cause mottling or distortion. Inoculations back to Turkish tobacco from the unrubbed leaves of mosaic-affected plants were positive except for severe mosaic type 1 and ring mosaic, indicating that systemic infection occurs with the mosaics.

Etch, etch +, severe etch, and etch (Georgia) produce on *P. heterophylla* a mottling and slight rugoseness almost identical with the symptoms produced by tobacco mosaics on tobacco. There is some distortion of the leaves and slight stunting of the plants affected with etch +, and those affected with severe etch are slightly more distorted and stunted. Etch and etch (Georgia) cause no stunting and distortion. Transfers to

TABLE 12
Inoculations of a Perennial Groundcherry in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.[15]	Number positive	Days to first symptoms	Inoculation back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	2	2	7	2	0	.....	.....
				2	0	.....	.....
Mild mosaic type 1	2	2	7	2	1	12	Mild mosaic type 1
				1	0	.....	.....
Severe mosaic type 2	2	2	7	2	2	8	Severe mosaic type 2
Mild mosaic type 2	2	2	7	2	2	5–8	Mild mosaic type 2
Yellow mosaic	2	2	7	2	1	7	Yellow mosaic
				1	1	7	Yellow mosaic
White mosaic	2	2	7	2	2	7–8	White mosaic
Ring mosaic	2	2	7	2	0	.....	.....
				2	0	.....	.....
Etch	2	2	17	2	1	4	Etch
Etch (Ga.)	2	2	17	2	2	7–8	Etch (Ga.)
Etch+	2	2	17	2	2	4–6	Etch+
Severe etch	2	2	17	2	2	4–8	Severe etch
Veinbanding	2	?	?	2	2	7	Veinbanding
Coarse etch	2	2	17	2	1	23	Coarse etch
				2	0	.....	.....
Ringspot	2	1?	29	2	0	.....	.....
Cucumber mosaic type 1	4	?	12	4	2	7–8	Cucumber mosaic type 1
				2	0	.....	.....
Cucumber mosaic type 2	2	2	17	2	1	6	Cucumber mosaic type 2
Cucumber mosaic type 3	2	2	17	2	0	.....	.....
				2	2	10–12	Cucumber mosaic type 3
"Healthy potato" virus	2	2	29	2	2	27	"Healthy potato" virus
				2	0	.....	.....
"Healthy potato" virus+ veinbanding	2	2	11	2	2	7–15	"Healthy potato" virus+ veinbanding
Cucumber mosaic type 3+veinbanding	2	2	17	2	0	.....	.....

[15]The groundcherry plants were inoculated May 14, 1929. The transfers back to Turkish tobacco were made July 2, 1929.

Table 12—Continued

Virus type	Number plants inoculated.[16]	Number positive	Days to first symptoms	Inoculation back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Checks. Rubbed with juice from healthy tobacco	3	0	.....	2	2	12–15	Cucumber mosaic type 3+veinbanding
				2	0	.....	.....

tobacco from the unrubbed leaves of plants affected with these 4 viruses were positive.

Coarse etch-inoculated plants develop chlorotic spots between the veins in about 17 days. Several weeks after inoculation, chlorotic spots are visible along the veins, but they are never prominent. Necrotic lines sometimes develop along the veins. After these leaves have fallen, the plants appear normal. Transfers to tobacco from unrubbed leaves of coarse etch-affected plants were positive.

The 3 cucumber mosaics produce symptoms of the same general type on this species. About 15 days after inoculation, the leaves at the growing point are narrow and chlorotic and have occasional blister-like or puffed areas. These leaves have a mottling like that of plants affected with the etch viruses. These symptoms are not very striking in plants affected with cucumber mosaic type 1, slightly more noticeable in plants having cucumber mosaic type 2, and marked in plants affected with cucumber mosaic type 3. In the latter the leaves are narrow and malformed and the plants are badly stunted. No difference was noted in plants inoculated with cucumber mosaic type 3 alone and this in combination with veinbanding. Inoculations back to tobacco from the cucumber mosaic-affected plants were positive.

Plants inoculated with "healthy potato" virus develop pale green areas along the veins of unrubbed leaves in a month

to 6 weeks after inoculation. A faint mottling, consisting of numerous very small, pale green spots, develops in the vein islets. Plants affected with this virus probably would be considered healthy unless comparisons could be made with healthy controls. Plants affected with cucumber mosaic type 3 + vein-banding had symptoms identical to those affected with "healthy potato" virus alone.

The ringspot inoculations to this species were unsatistory. Only two plants were used in this test, but each plant was inoculated twice. In one plant fairly distinct ring-like chlorotic patterns developed in the new growth but a transfer from this plant to tobacco gave no symptoms in the latter.

To Physalis pubescens. The results of inoculations of *P. pubescens* with viruses are given in Table 13. Severe and mild mosaics and ring mosaic produce symptoms on this annual species of Physalis that are almost identical. Chlorotic spots may appear on rubbed leaves in 5 to 10 days or these may not develop, in which case the first symptom is a faint mottling of the new leaves. Distortion in the form of rolled margins and twisting of the leaves occurs in the new growth.

Yellow mosaic produces yellow areas between the veins in addition to the mottling and distortion caused by the other mosaics. Yellow mosaic plants have a general chlorotic appearance and the leaves are crinkled.

White mosaic plants develop small, chlorotic spots on rubbed leaves about 4 days after inoculation. Unrubbed leaves, 7 days after inoculation, have prominent vein clearing and a crinkled appearance. Ten days after inoculation, the new leaves are devoid of green color except for an occasional green blister. Necrosis develops at about this time on the lower leaves, especially on rubbed ones. White mosaic causes greater injury to this species than any of the other viruses tested.

Etch, etch +, severe etch, and etch (Georgia), except for a difference in prominence, produce the same symptoms on this species. Five days after inoculation, the leaves at the growing point show vein clearing and a warty appearance. As the newer leaves form they become slightly distorted, narrow bands of pale green tissue appear along the veins and the leaves remain

TABLE 13

Inoculations of an Annual Groundcherry in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.[16]	Number positive	Days to first symptoms	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	2	2	5	2	2	3–5	Severe mosaic type 1
Mild mosaic type 1	2	2	10	2	2	5	Mild mosaic type 1
Severe mosaic type 2	2	2	7–9	2	2	4	Severe mosaic type 2
Mild mosaic type 2	2	2	5	2	2	3–5	Mild mosaic type 2
Yellow mosaic	2	2	5	2	2	5	Yellow mosaic
White mosaic	2	2	4–5	2	2	3	White mosaic
Ring mosaic	2	2	7	2	2	3	Ring mosaic
Etch	2	2	5	2	2	5	Etch
Etch (Ga.)	2	2	7	2	0	.....	.....
Etch+	2	2	5	2	2	5	Etch+
Severe etch	2	2	5	2	2	5–8	Severe etch
Veinbanding	2	2	15	2	2	7	Veinbanding
Coarse etch	2	2	7	2	2	7	Coarse etch
Ringspot	2	0	.....	2	0	.....	.....
	2	0	.....	0	....	.....	.....
Cucumber mosaic type 1	4	4	5–7	4	4	8–14	Cucumber mosaic type 1
Cucumber mosaic type 2	2	2	5–7	2	1	6	Cucumber mosaic type 2
Cucumber mosaic type 3	2	2	5	2	2	7	Cucumber mosaic type 3
"Healthy potato" virus	2	2	7	2	2	7	"Healthy potato" virus
"Healthy potato" virus+ veinbanding	2	2	3	2	1	11	Veinbanding
Cucumber mosaic type 3+veinbanding	2	2	5	2	1	7	Cucumber mosaic type 3+veinbanding
Checks. Rubbed with juice from healthy tobacco	2	0	.....	2	0	.....	.....

[16]The groundcherry plants were inoculated June 10, 1929. The inoculations back to Turkish tobacco were made June 25, 1929.

small. These symptoms increase in distinctness from etch thru etch + to severe etch.

The symptoms produced by coarse etch on this species are obscure. Seven days after inoculation, circular chlorotic spots with a dark green center appear on the new leaves. No other symptoms develop and the plants are not stunted.

Veinbanding produces a slight chlorosis that occurs as numerous small, pale spots along the veins, which give affected plants a speckled appearance. Affected plants are not stunted.

Four inoculations with the virus of ringspot to *P. pubescens* were negative.

Cucumber mosaics types 1 and 3 and cucumber mosaic type 3 + veinbanding cause, on this species, a clearing of the veins and a wrinkling of the leaf surfaces about 7 days after inoculation. Leaves developing 10 days after inoculation are narrow, wrinkled and distorted, and pale green to almost yellow tissue appears between the veins. Plants affected with these viruses are stunted. All these symptoms are more prominent in cucumber mosaic type 3 than in cucumber mosaic type 1 plants. No differences are apparent in plants affected with cucumber mosaic type 3 + veinbanding and those affected by cucumber mosaic type 3 alone.

Cucumber mosaic type 2 causes, in addition to the vein clearing and wrinkling of the leaves, pale green almost yellow areas along the veins, while the interveinal areas remain normal green. No distortion of older leaves occurs.

The principal symptom produced by "healthy potato" virus on *P. pubescens* is a faint mottling on rubbed leaves as a slight chlorosis in the vein islets. Plants affected with "healthy potato" virus + veinbanding are identical to those affected by "healthy potato" virus alone. Healthy controls are necessary for comparison in order to recognize the symptoms.

All the transfers to tobacco from unrubbed leaves of affected plants were positive for their respective types except those of etch (Georgia) which were negative.

To horsenettle (Solanum carolinense). The results of the inoculations of horsenettle with viruses are given in Table 14. Severe mosaics, types 1 and 2, cause a slight rugoseness and a mosaic-like mottling on the leaves. The first symptom, which

TABLE 14
Inoculations of Horsenettle in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.[17]	Number positive	Days to first symptoms	Inoculation back to Turkish tobacco			
				No. plants inoculated	Number positive	Days to first symptoms	Type transmitted.
Severe mosaic type 1	1	1	12	4	4	7–8	Severe mosaic type 1
Mild mosaic type 1	1	1	18	4	2	7–9	Mild mosaic type 1
Severe mosaic type 2	1	1	12	4	4	7–9	Severe mosaic type 2
Mild mosaic type 2	1	1	12	4	4	7–9	Mild mosaic type 2
Yellow mosaic	1	1	12	4	4	5–9	Yellow mosaic
Ring mosaic	1	2	12	4	4	10–16	Ring mosaic
Etch[18]	1	1	20	6	0	.....	.....
	1	0	.....	0	....	.....	.....
Etch+	1	1	16	3	0	.....	.....
	1	0	.....	0	....	.....	.....
Severe etch	1	1	?	2	1	25	Severe etch
Veinbanding	1	0	.....	0	....	.....	.....
Coarse etch	1	0	.....	3	0	.....	.....
	1	0	.....	0	....	.....	.....
Ringspot	2	0	.....	2	0	.....	.....
	4	0	.....	0	....	.....	.....
	4	0	.....	0	....	.....	.....
Cucumber mosaic type 1	2	2	26	4	1	11	Cucumber mosaic type 1
Cucumber mosaic type 2	1	1	26	2	0	.....	.....
"Healthy potato" virus	3	3	23–25	16	12	7–25	"Healthy potato" virus
"Healthy potato" virus 1+ veinbanding	1	1	25	8	4	7–18	"Healthy potato" virus
Cucumber mosaic type 3+veinbanding	1	1	25	6	2	11	Cucumber mosaic type 3
Check. Rubbed with juice from healthy tobacco	1	0	.....	4	0	.....	.....

[17]The inoculations of horsenettle were made in March, June and July. The inoculations back to Turkish tobacco were made in 1 month to 13 months after inoculation of the horsenettle.

[18]Aphids were transferred from this plant to tobacco and etch developed in the latter.

appears about 12 days after inoculation, is rugoseness of the bud leaves. This is followed, 3 to 4 days later, by mottling.

The 2 mild mosaics cause mottling but affected plants develop no rugoseness.

The early symptoms produced by yellow mosaic are similar to those of the 2 severe mosaics. About 3 weeks after inoculation, ring-like areas ⅛ to ¼ inch in diameter appear on the newer leaves. These rings have a dark green center which is surrounded by a band of yellow tissue.

Plants affected with ring mosaic develop brown, necrotic, curved patterns along the veins and midribs of the leaves in addition to the mottling and blistering produced by the severe mosaics.

The symptoms produced by etch, etch +, and severe etch are obscure. Affected plants are always stunted and have irregular chlorotic areas along the veins of the slightly wrinkled young leaves.

Repeated attempts to produce infection on horsenettle with ringspot, veinbanding, and coarse etch were unsuccessful and transfers from these plants to tobacco were negative.

Cucumber mosaics, types 1 and 2, produce small chlorotic spots both on rubbed and unrubbed leaves of horsenettle. These spots are inconspicuous and unless comparisons are made with healthy controls they would probably not be noticed.

Cucumber mosaic type 3 + veinbanding plants have, in addition to the chlorotic spots described for the other cucumber mosaics, narrow and distorted bud leaves. Irregular, dark green, slightly raised areas develop along the veins of these leaves. Only cucumber mosaic type 3 was obtained when a transfer was made to tobacco.

"Healthy potato" virus and "healthy potato" virus + veinbanding produce, on horsenettle, an inconspicuous mottling characterized by small chlorotic spots between the veins. Transfers to tobacco from plants affected with "healthy potato" alone or in combination with veinbanding gave only "healthy potato."

There was no difficulty in transferring any of the tobacco mosaics from horsenettle to tobacco by the rubbing method and

each came true to type. Six mechanical transfers to tobacco from the etch plant were negative. A colony of aphids which appeared on this plant was placed on 3 tobacco plants and etch developed in the latter. Three mechanical transfers to tobacco from the etch + plant were negative, and only 1 tobacco plant of 4 inoculated from the severe etch horsenettle developed severe etch. Six transfers were made to tobacco from the cucumber mosaic type 1 plant and only 1 infection developed, and no infection resulted from the 4 transfers to tobacco from the cucumber mosaic type 2 plant.

In view of the difficulty of transferring some of the viruses from horsenettle to tobacco by the rubbing method, it may be that those viruses which appeared not to infect horsenettle might occur in this species under natural conditions and might be transmitted by insects. Since the symptoms produced by most of the viruses, other than tobacco mosaic, are obscure, affected plants might be easily overlooked in the field.

To jimson (*Datura stramonium L*). The results of inoculating jimson with tobacco viruses are given in Table 15. All the tobacco mosaics, regardless of type, produce the same symptoms on jimson. In 3 to 11 days, inoculated plants develop necrotic circular spots $\frac{1}{16}$ to $\frac{1}{4}$ inch in diameter on rubbed or pricked leaves. A week later, necrotic streaks develop on the midribs and petioles of these leaves and usually spread down the stem (Plate XX, Figure 8). The rubbed leaves gradually shrivel and fall. Infection seems almost entirely local, as unrubbed leaves have shown necrosis but once. The results of transfers back to tobacco were always negative when leaves of jimson which showed no necrosis were used as inoculum. In the transfers to tobacco from mosaic-affected jimson, the time to the appearance of symptoms in tobacco is rather long, suggesting that infection may have come from the original inoculum on the rubbed leaves.

Etch, etch +, severe etch and etch (Georgia) produce rather striking symptoms on jimson. The first sign of infection appears in 1 to 3 weeks after inoculation as vein clearing of the new leaves. A few days later, the growing point leaves are chlorotic, twisted, and abnormally narrow. All the etch viruses produce symptoms which increase in severity from etch thru

TABLE 15

Inoculations of Jimson in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive.	Average number days to first symptoms.	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Average number days to first symptoms.	Type transmitted.[19]
Severe mosaic type 1	7	7	6	3	1	22	Severe mosaic type 1
Mild mosaic type 1	4	4	7	1	1	11	Mild mosaic type 1
Severe mosaic type 2	7	7	5	6	3	15	Severe mosaic type 2
Mild mosaic type 2	6	6	6	6	2	16	Mild mosaic type 2
Yellow mosaic	7	7	5	4	2	12	Yellow mosaic
Ring mosaic	7	7	7	4	1	9	Ring mosaic
Etch	7	7	9	4	4	10	Etch
Etch (Georgia)	5	3	17	3	3	13	Etch (Georgia)
Etch+	7	6	9	6	4	9	Etch+
Severe etch	8	4	8	4	3	10	Severe etch
Veinbanding	6	0	0	2	0	.....	.....
Coarse etch	7	4	7	4	4	10	Coarse etch
Ringspot	6	5	7	3	2	8	Ringspot
Cucumber mosaic type 1	14	4(?)	20	12	0	.....	.....
Cucumber mosaic type 2	7	3	11	6	2	9	Cucumber mosaic type 2
Cucumber mosaic type 3	5	3	21	3	1	13	Cucumber mosaic type 3
"Healthy potato" virus	14	13	8	6	6	10	"Healthy potato" virus
"Healthy potato" virus+ veinbanding	7	7	8	10	10	10	"Healthy potato" virus, 8, and 2 "Healthy potato" virus +veinbanding
Cucumber mosaic type 3+veinbanding	7	3	17	6	1	8	Cucumber mosaic type 3
Checks. Rubbed with juice of healthy tobacco	10	0	.....	4	0	.....	.....

[19]In all the transfers to tobacco where tobacco mosaic resulted necrotic leaves were used as inoculum.

etch + to severe etch. Leaves of etch + plants are sometimes little more than midribs. The margins of upper leaves of severe etch plants are rolled upward and meet over the midribs, and

the lower leaves are chlorotic except for narrow bands of green tissue along the veins. The flowers of severe etch plants die soon after they form. Transfers to tobacco from unrubbed leaves of these plants were positive, indicating that the infections were systemic.

Coarse etch plants have a mottling which consists of pale green tissue along the veins of unrubbed leaves. These areas gradually disappear and in 3 months after inoculation the plants appear almost healthy. Transfers to tobacco from the unrubbed leaves of coarse etch plants were positive.

Six attempts to transfer the virus of veinbanding to jimson appeared to be negative.

Within 5 to 7 days after inoculation, ringspot produces small, circular, water-soaked spots with brown centers, on rubbed leaves of jimson. Small rings and spots appear on unrubbed leaves and gradually increase in size until the leaves are killed. Rings appear also on the calyx tubes. After 10 weeks growth, little remains but a tuft of small, pale green leaves at the growing point. In these tests ringspot plants did not develop seed pods. Transfers to tobacco resulted in ringspot.

Jimson plants inoculated with cucumber mosaics, types 1 and 3, develop only local symptoms consisting of a few small chlorotic spots on rubbed leaves. The results of transfers to tobacco from cucumber mosaic type 1 plants were negative but one infection was obtained in 3 transfers to tobacco from the cucumber mosaic type 3 plant, indicating that jimson is a possible host of the cucumber mosaics. The symptoms produced by cucumber mosaic type 3 + veinbanding were identical to those of the cucumber mosaics alone.

"Healthy potato" virus and this in combination with veinbanding, produce small, necrotic spots rarely larger than $\frac{1}{16}$ inch in diameter, on rubbed leaves, about 6 days after inoculation. Mottling, as pale green tissue between the veins and dark areas along the veins, appears 3 to 7 days later. This mottling, which is even visible on the calyx tubes, is present until maturity. The affected plants are not stunted and produce normal fruits. "Healthy potato" virus only was obtained in 8 out of 10 transfers to tobacco from jimson affected with this virus

alone or in combination with veinbanding. In 2 transfers from jimson affected with the combination virus disease only "healthy potato" virus was obtained, but in transfers from these to other tobacco plants, the combination disease ("spot-necrosis") resulted in the tobacco.

To cucumber (*Cucumis sativus*). The results of attempts to transfer a number of the virus diseases to cucumber are given in Table 16. Six to 7 plants were grown in tumblers and inoculations made when the plants had formed the first true leaf. Two methods of inoculation were used. The first consisted in placing a water extract of virus-diseased tobacco on the cotyledons of the cucumber plants and pricking thru this in 10 to 20 places with 00 insect pins. The second method consisted in rubbing the fresh virus extract on the cotyledons and first leaves by means of the sterile swabs. Both methods gave positive results with the cucumber mosaics and ringspot, but the rubbing method was discontinued because it caused considerable injury to the tender foliage, and local symptoms were obscured. The results of the prick inoculations are given in Table 16, in which it is shown that infection resulted on cucumber with only cucumber mosaics and ringspot.

Ringspot cucumber plants develop small, chlorotic spots, ⅛ inch in diameter, either on the pricked cotyledons or on the first leaves, in 3 to 20 days. Some of these spots on the leaves increase in size until they attain a diameter of ½ inch. The spots may be composed of alternate bands of yellow and green tissue, the yellowed portion of which may and usually does become necrotic (Plate XX, Figures 4 and 6).

Plants affected with ringspot were set in 4-gallon jars and grown to maturity. Chlorotic spots continued to develop but these ringspot plants were not so badly distorted and stunted as those affected with the cucumber mosaics. Chlorotic rings ⅛ to ½ inch in diameter developed on the fruit. The latter showed no tendency to form white pickle and they were not malformed.

Cucumber mosaics types 1 and 3, produce symptoms on cucumber that differ in severity only. The first symptoms appear, usually in 6 to 16 days, as pale green, indefinite spots at the bases of leaves that develop after inoculation.

TABLE 16

Inoculations of Cucumber in the Greenhouse with Tobacco Viruses

Virus type	Number plants inoculated.	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	38	0	.....	0	....	.....	.....
Mild mosaic type 1	38	0	.....	3	0	.....	.....
Mild mosaic type 2	36	0	.....	3	0	.....	.....
Severe mosaic type 2	37	0	.....	1	0	.....	.....
Yellow mosaic	42	0	.....	3	0	.....	.....
Ring mosaic	42	0	.....	1	0	.....	.....
Etch	38	0	.....	3	0	.....	.....
Etch+	34	0	.....	3	0	.....	.....
Severe etch	28	0	.....	3	0	.....	.....
Veinbanding	6	0	.....	2	0	.....	.....
Coarse etch	31	0	.....	3	0	.....	.....
Ringspot	52	12	6–10	0	....	.....	.....
Cucumber mosaic type 1	51	14	7–10	0	....	.....	.....
Cucumber mosaic type 3	24	10	7–13	0	....	.....	.....
Cucumber mosaic type 3+veinbanding	13	3	12–13	2	2	6	Cucumber mosaic type 3
Checks. Inoculated with juice from healthy tobacco	76	0	.....	2	0	.....	.....

Cucumber mosaic-affected plants were set in 4-gallon jars and grown until mature. The spotted leaves and those appearing later gradually developed the mottling described by Doolittle (6), the margins rolled inward and downward and frequently the leaves wilted and died. Both types caused stunting of the plants, decreased size of the leaves, and white pickle. They differed in that cucumber mosaic type 3 produced all these symptoms more prominently than type 1.

Cucumber mosaic type 3 + veinbanding resulted in symptoms identical to those caused by the former alone. Inoculations to tobacco from these plants gave cucumber mosaic type 3 only and inoculations from veinbanding plants were negative.

Cucumber mosaic type 2 was not tested in the greenhouse but was in the field, where the symptoms were similar to those of type 1. Type 2 caused greater injury.

Inoculations to tobacco were made from all plants except those affected with the cucumber mosaics and ringspot, and all were negative. As the symptoms of ringspot and the cucumber mosaics were striking and as, in former work, there had been no difficulty in transferring these viruses to tobacco, it was thought unnecessary to make the transfers in order to establish infection.

To pokeweed (*Phytolacca decandra*). Pokeweed is commonly affected with a mosaic disease in Kentucky. Such plants are mottled like tobacco plants affected with tobacco mosaic. Numerous unsuccessful attempts have been made to transfer this disease to tobacco. It was thought that inoculations from known viruses to pokeweed might give some idea of the nature of the naturally-occurring mosaic. The results of these inoculations are given in Table 17. Of the types tested, negative results were obtained with all viruses except cucumber mosaics, types 1, 2 and 3, coarse etch, ringspot, and "healthy potato" virus. Of these types only the cucumber mosaics and ringspot seemed to cause systemic infection.

All the cucumber mosaics produce the same symptoms on pokeweeed and these are entirely different from those observed in naturally-infected plants. The first symptoms appear about 7 days after inoculation as small chlorotic spots on rubbed leaves. These spots gradually increase in size by the production of alternate rings of chlorotic and pale green tissue. The unrubbed leaves, after variable periods, either develop these rings or small oakleaf patterns along the veins. The surfaces of these leaves are wrinkled, and they droop markedly. Plants affected with the cucumber mosaics have a general chlorotic appearance and are dwarfed.

Seven naturally-infected pokeweed plants were collected on May 5, 1928, and inoculations were made to tobacco, cucumber, apple of Peru (*Nicandra physalodes*), jimson, and to healthy pokeweed, all of which species are susceptible to the cucumber mosaics. None of the species inoculated with the 7 samples of pokeweed mosaic extract became infected except the pokeweed

TABLE 17

Inoculations of Pokeweed in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.[20]	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	2	0	.....	1	0	.....	.....
Mild mosaic type 1	1	0	.....	1	0	.....	.....
Severe mosaic type 2	2	0	.....	1	0	.....	.....
Mild mosaic type 2	1	0	.....	0	....	.....	.....
Yellow mosaic	2	0	.....	1	0	.....	.....
White mosaic	1	0	.....	0	....	.....	.....
Ring mosaic	2	0	.....	1	0	.....	.....
Etch	2	0	.....	1	0	.....	.....
Etch (Ga.)	1	0	.....	0	....	.....	.....
Etch+	2	0	.....	1	0	.....	.....
Severe etch	2	0	.....	1	0	.....	.....
Veinbanding	1	0	.....	1	0	.....	.....
Coarse etch	2	1	5	1	0	.....	.....
Ringspot	2	2	7 8	3	0	.....	.....
Cucumber mosaic type 1	4	4	7 8	2	2	13	Cucumber mosaic type 1
Cucumber mosaic type 2	2	2	6 8	1	1	14	Cucumber mosaic type 2
Cucumber mosaic type 3	1	1	8	0	....	.....	.....
"Healthy potato" virus	3	2	30	3	0	.....	.....
"Healthy potato" virus+ veinbanding	2	1	10	1	0	.....	.....
Cucumber mosaic type 3+veinbanding	2	2	7–8	1	0	.....	.....
Pokeweed mosaic from pokeweed (naturally infected)	2	2	12	0	....	.....	.....
Checks. Rubbed with juice from healthy tobacco	4	0	.....	2	0	.....	.....

[20]The inoculations of pokeweed were made March 27, 1928 and the transfers back to Turkish tobacco were made April 20, 1928.

which developed symptoms typical of the naturally-occurring mosaic. There has been no difficulty in transferring to tobacco, cucumber mosaic from pokeweed affected with known types of this virus. Since the writer has been unable to transfer pokeweed mosaic from naturally-infected plants to a number of species susceptible to cucumber mosaic, and since the symptoms produced in pokeweed by inoculation with the cucumber mosaics are different from pokeweed mosaic as it occurs in Kentucky, it is believed that the pokeweed mosaic of common occurrence in this State is not cucumber mosaic.

Ringspot produces necrotic rings ⅛ to ¼ inch in diameter on the rubbed leaves of pokeweed. Rings of this type develop somewhat later in great numbers on unrubbed leaves and continue to form thruout the life of the plant. Attempts to transfer ringspot to tobacco from pokeweed have been unsuccessful. Wingard (36) obtained infection on tobacco from ringspot pokeweed and suggests the latter as a possible overwintering agency of this disease. Ringspot-diseased pokeweed has not been observed by the writer in the field in Kentucky.

Coarse etch produces the same type of necrotic patterns on pokeweed as it does on tobacco but on rubbed leaves only. Attempts to transfer coarse etch to tobacco from pokeweed, using unrubbed leaves as inoculum, have been unsuccessful.

"Healthy potato" virus produces small, chlorotic spots on rubbed leaves as the only symptom. The infection seems to be entirely local because transfers to tobacco from unrubbed leaves were negative.

To eggplant (*Solanum melongena* L. var. *esculentum* Nees). Eggplants were set in half-gallon jars of fertile soil and were inoculated when 4 inches high. The results of these inoculations are given in Table 18. The tobacco mosaics produced local infections in the form of small, brown, necrotic spots, in 3 to 5 days. After a time, usually 15 to 20 days, necrotic streaks up to an inch in length appeared on the midveins and veins of rubbed leaves. There was never any mottling and after 2 months growth, aside from their slightly smaller size and absence of lower leaves, affected plants seemed normal. Transfers to tobacco from unrubbed leaves were negative.

The inoculations to eggplant with etch, etch +, severe etch,

and ringspot were negative. Wingard 36, was able to transmit ringspot to eggplant but was unable to transfer the disease back to tobacco.

In 15 days coarse etch-inoculated plants develop irregular patterns of dark green tissue along the veins of unrubbed leaves. This mottling continues to develop in the leaves as they form but no necrosis was observed 2 months after inoculation. Two transfers from coarse etch plants were negative.

Veinbanding produced no symptoms on eggplant in this test, but 1 of 2 transfers back to tobacco was positive for this type.

Cucumber mosaic type 3 and this in combination with veinbanding produce the same symptoms on eggplant. In 19 to 25 days after inoculation, the new leaves develop a mottling and puffing similar to the symptoms produced on tobacco by the cucumber mosaics. Then or a few days later, necrotic patterns develop along the veins of rubbed leaves. Transfers to tobacco from unrubbed leaves of eggplant affected with cucumber mosaics were positive.

"Healthy potato" virus and this in combination with veinbanding produce no symptoms on eggplant. Two of 4 transfers to tobacco from "healthy potato" virus-affected plants were positive for this type, but all the transfers from the "healthy potato" virus + veinbanding plants were negative.

To Nicotiana rustica. Plants of *Nicotiana rustica* were grown in tumblers and inoculated when they had about 6 leaves. The results of the inoculations are given in Table 19. Six tobacco mosaics produced the same symptoms on this species. Small, brown, circular, water-soaked spots develop on rubbed leaves in 6 to 13 days after inoculation. A few days later, similar spots develop on unrubbed leaves. These spots increase in size rapidly and kill the affected leaves. A few small, distorted flowers with necrotic spots on the calyx tubes form, but the plants seldom produce more than 3 or 4 undersized, distorted seed pods. Infected plants are markedly stunted.

Etch and etch + produce a mosaic-like mottling and cause some distortion and stunting. Necrosis does not develop in greenhouse plants.

Plants inoculated with coarse etch develop prominent chlorotic patterns along the veins of both rubbed and unrubbed

TABLE 18

Inoculations of Eggplant in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.[21]	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco			
				No. plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	2	2	4	2	0	.....	.....
Mild mosaic type 1	2	2	4	2	0	.....	.....
Severe mosaic type 2	2	2	4–5	2	0	.....	.....
Mild mosaic type 2	2	2	4	2	0	.....	.....
Yellow mosaic	2	2	5	2	0	.....	.....
Ring mosaic	2	2	4	2	0	.....	.....
Etch	2	0	.....	2	0	.....	.....
Etch+	2	0	.....	2	0	.....	.....
Severe etch	2	0	.....	2	0	.....	.....
Veinbanding	2	?	.....	2	1	19	Veinbanding
Coarse etch	2	1	15	2	0	.....	.....
Ringspot	4	0	.....	2	0	.....	.....
Cucumber mosaic type 1	4	4	19	4	4	16	Cucumber mosaic type 1
"Healthy potato" virus	6	?	?	4	2	19	"Healthy potato" virus
"Healthy potato" virus+ veinbanding	2	0	.....	2	0	.....	.....
Cucumber mosaic type 3+veinbanding	2	2	?	2	1	19	Cucumber mosaic type 3
Checks. Rubbed with juice from healthy tobacco	2	0	.....	2	0	.....	.....

[21]The eggplants were inoculated May 3 and the transfers back to Turkish tobacco were made June 29. Inoculum from unrubbed leaves.

leaves in 7 to 8 days. Coarse etching, as described on tobacco, appears in about a week and later chlorotic patterns, like those described in tobacco, develop in the new leaves.

Veinbanding plants develop small, chlorotic spots in the interveinal areas in about two weeks after inoculation.

Ringspot produces the same type of rings on *N. rustica* as on tobacco. The first rings appear as local infections in 4 to 7

days after inoculation. Chlorotic rings of variable sizes are produced thruout the life of the plant, but they either become necrotic slowly or not at all.

Plants inoculated with cucumber mosaic type 1 and cucumber mosaic type 3 + veinbanding appear normal for almost a month after inoculation. The growing point leaves become chlorotic and slightly distorted at this time. The leaves that develop after this time have the puffed areas and the same type of distortion as tobacco plants affected with the cucumber mosaics. The flowers are reduced in size and number and some of them are choripetalous, whereas the normal flowers are sympetalous.

TABLE 19

Inoculations of Nicotiana Rustica in the Greenhouse with Tobacco Viruses.

Virus type	Number plants inoculated.[22]	Number positive.	Days to first symptoms.
Severe mosaic type 1	3	3	6
Mild mosaic type 1	2	2	6–13
Severe mosaic type 2	2	2	6
Mild mosaic type 2	2	2	6–13
Yellow mosaic	2	2	6–14
White mosaic	1	1	6
Ring mosaic	1	1	7
Etch	4	4	8–12
Etch+	4	4	6–12
Veinbanding	3	3	8–13
Coarse etch	3	2	8
Ringspot	3	3	5–16
Cucumber mosaic type 1	4	3	25
Cucumber mosaic type 3+ veinbanding	2	2	26
Checks. Rubbed with juice from healthy tobacco	3	0	.....

[22]These inoculations were made December 19, 1927. Transfers back to Turkish tobacco were not made from these plants. These inoculations were repeated in 1929 and transfers from all types to tobacco resulted in infection.

To apple of Peru (Nicandra physalodes). The results of inoculating apple of Peru with tobacco viruses are given in Table 20. All the tobacco mosaics tested produce the same symptoms on apple of Peru. Nine days after inoculation, chlo-

rotic spots $\frac{1}{8}$ to $\frac{1}{2}$ inch in diameter develop on rubbed leaves. These leaves gradually become yellow and the spots are outlined by a characteristic green border. This border sometimes becomes necrotic, but the yellowed part of the spot does not die except for a small area in the center. Unrubbed leaves develop no symptoms. The rubbed leaves gradually die and fall.

Etch, etch +, severe etch, and etch (Georgia) produce the same general type of symptoms. No definite symptoms appear until about 15 to 20 days after inoculation, at which time the young leaves show a mottling consisting of pale green tissue along the veins. Then, or a few days later, numerous tiny necrotic spots appear on the unrubbed leaves. Plants affected with etch and etch (Georgia) are noticeably stunted, and those affected with etch + and severe etch are markedly stunted.

Coarse etch plants develop pinhead necrotic spots on rubbed leaves 8 days after inoculation. After variable periods, pale green, water-soaked areas develop around the latter, and these borders become parchment-like. Necrotic spots develop on the calyx tubes at the time the flowers open. Coarse etch plants are not stunted.

Plants inoculated with veinbanding show no evidence of infection, and transfers from these to tobacco are always negative.

Ringspot produces small chlorotic and necrotic rings on rubbed leaves about 15 days after inoculation. No other symptoms develop.

The three cucumber mosaics produce the same symptoms. Chlorotic and necrotic spots similar to those produced by the tobacco mosaics on this species appear on rubbed leaves in 7 to 15 days, but cucumber mosaics, unlike the tobacco mosaics, produce these spots on the unrubbed leaves as well. Necrosis also appears on the petioles and stems as narrow streaks often $1\frac{1}{2}$ inches long. The leaves at the growing point are narrow and chlorotic and the plants are stunted.

Cucumber mosaic type 3 + veinbanding causes the same general type of symptoms as cucumber mosaic type 3 alone, but in more severe form. The leaves of affected plants are more narrow, more chlorotic, and there is more necrosis and stunting.

TABLE 20

Inoculations of Apple of Peru in the Greenhouse with Tobacco Viruses

Virus type	Number plants inoculated.	Number positive.	Average number of days to first symptoms.	Inoculations back to Turkish tobacco			
				Number plants inoculated.	Number positive	Average number of days to first symptoms.	Type transmitted.
Severe mosaic type 1	5	4	8	2	2	9	Severe mosaic type 1
Mild mosaic type 1	5	4	9	2	0	.....	.....
Severe mosaic type 2	5	4	8	2	2	7	Severe mosaic type 2
Mild mosaic type 2	5	4	8	2	0	.....	.....
Yellow mosaic	5	1	7	2	0	.....	
White mosaic	2	2	9	2	0	.....	
Ring mosaic	4	2	12	2	0	.....	.....
Etch	4	3	13(?)	2	2	9	Etch
Etch (Georgia)	2	2	9	2	2	9	Etch (Georgia)
Etch +	3	3	13(?)	2	2	9	Etch +
Severe etch	2	0	.....	0	...	.....	.....
Veinbanding	4	0	.....	2	0	.....	.....
Coarse etch	4	3	9	2	2	9	Coarse etch
Ringspot	4	4	17	4	2	5	Ringspot
Cucumber mosaic type 1	4	2	15	4	4	9	Cucumber mosaic type 1
Cucumber mosaic type 2	3	3	13	2	2	9	Cucumber mosaic type 2
Cucumber mosaic type 3	2	2	9	2	2	17	Cucumber mosaic type 3
"Healthy potato" virus	10	10	13	4	3	19	"Healthy potato" virus
"Healthy potato" virus + veinbanding	4	4	8	2	2	16	"Healthy potato" virus
Cucumber mosaic type 2 + veinbanding	4	4	11	2	0	.....	.....
Checks. Rubbed with juice from healthy tobacco	6	6	.....	2	0	.....	.....

In two inoculations a different type of the plants were almost dead a month after inoculation.

"Healthy potato" virus produces small, necrotic spots or [illegible] on [illegible] leaves 7 to 12 days after inoculation. Some time later the [illegible] turn yellow and curved like pa-

terns, with green borders, appear along the veins of the leaves. These borders sometimes become necrotic. The leaves at the growing point develop a mosaic-like mottling and rugoseness and affected plants are slightly stunted. "Healthy potato" virus + veinbanding causes plants to develop the same symptoms as those inoculated with "healthy potato" virus alone.

Transfers were made to tobacco from the unrubbed leaves of the inoculated plants. The results of the inoculations from mosaic plants were not all positive, which may mean that systemic infection does not always occur with the mosaics. All other transfers gave symptoms in tobacco typical of the expected type except veinbanding.

FIELD INOCULATIONS

It was thought advisable to repeat the greenhouse inoculations with some of the species in the field and to supplement them with plants not inoculated in the greenhouse. During the summer of 1928, inoculations were made by the sterile swab method to Burley tobacco, tomato, pepper, cucumber, muskmelon, *N. rustica*, and a number of other cultivated plants.

To Burley tobacco. The results of the inoculations to Burley tobacco are given in Table 21. The plants, grown from greenhouse seed, were pulled by the writer after thoroly washing the hands with soap and water and set with a setter June 13, 1928. The inoculations were made on July 26, 1928, with diluted extracts of virus-diseased tobacco plants growing in the greenhouse. Before inoculation, each field plant was carefully inspected to see that no virus symptoms were present. Symptoms typical of each type resulted with all the viruses tested except cucumber mosaic type 3 + veinbanding and "healthy potato" + veinbanding. Transfers from these to tobacco plants gave veinbanding only.

The symptoms produced on Burley tobacco by the viruses tested have been described in the first part of this bulletin. The photographs in Plates I, II, III, V, VII, VIII, IX, X, XI, XII, and XIII are of the Burley plants in this test.

To tomato. The results of inoculating tomato are given in Table 22. Greenhouse-grown plants of the Stone variety,

pulled and set with clean pieces of paper in order not to introduce any virus disease from the hands, were set May 17, 1928. The inoculations were made June 15, 1928, by rubbing 2 leaves of each plant. As in the greenhouse, the two severe mosaics and ring mosaic caused more mottling and distortion than the two mild mosaics. It was necessary to examine the mild mosaic plants carefully in order to detect mottling. The lower leaves of plants affected with the severe mosaics and with ring mosaic developed irregular necrotic spots. Yellow mosaic plants, in addition to the mottling, necrosis, and distortion, had lemon-yellow tissue between the veins. This symptom was so striking that the affected row of plants could be distinguished from the others at a distance of several feet. Ring mosaic did not produce necrotic streaks on the leaf petioles and stems, and chlorotic rings were not observed on the fruit, as in the greenhouse.

The symptoms produced by the etch viruses, altho of the same type as those caused by these viruses in the greenhouse, were much more striking than in the latter. Tomato plants affected with any of these viruses might readily be confused with tobacco mosaic-diseased plants because of the mosaic-like mottling. Etch + and severe etch plants were distorted and stunted much more than those affected with the severe mosaics (Plate XVIII, Figures 1 and 2).

The results of the inoculations to tomato with viruses of ringspot, coarse etch, veinbanding, the cucumber mosaics and cucumber mosaic + veinbanding appeared negative.

"Healthy potato" virus caused necrosis in addition to the mottling described on greenhouse plants. This appeared 10 days after inoculation as numerous rusty-brown spots on rubbed and unrubbed leaves. No difference was observed in the symptoms of plants affected with "healthy potato" virus alone and this in combination with veinbanding.

In the transfers to tobacco in the greenhouse, the expected type was obtained in all cases except with "healthy potato" virus + veinbanding from which "healthy potato" only resulted.

To pepper. The results of inoculating bell pepper are given in Table 23. Greenhouse grown plants handled with the same care as the tomato plants were set May 17, 1928, and the inocu-

TABLE 21

Inoculations of Burley Tobacco in the Field with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco in greenhouse.			
				Number plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	35	34	9	2	2	5	Severe mosaic type 1
Mild mosaic type 1	35	34	9–12	2	2	5	Mild mosaic type 1
Severe mosaic type 2	37	36	9	2	2	5	Severe mosaic type 2
Mild mosaic type 2	35	35	9	2	2	7	Mild mosaic type 2
Yellow mosaic	34	34	9–11	2	2	5	Yellow mosaic
White mosaic	19	19	6	0	....	.....	.....
Ring mosaic	34	34	7	2	2	5	Ring mosaic
Etch	37	35	9–11	2	2	5	Etch
Etch+	37	37	9	2	2	5	Etch+
Severe etch	35	35	9	2	2	5	Severe etch
Veinbanding	30	29	11–15	2	2	7	Veinbanding
Coarse etch	35	3	16	2	0	.....	.....
Ringspot	35	18	9–27	2	1	9	Ringspot
Cucumber mosaic type 1	72	8	16–27	4	1	9	Cucumber mosaic type 1
Cucumber mosaic type 2	40	13	16–27	2	2	5	Cucumber mosaic type 2
"Healthy potato" virus	103	24	16	2	0	.....	.....
"Healthy potato" virus+ veinbanding	36	33[23]	13–17	2	2	9	Veinbanding
Cucumber mosaic type 3+veinbanding	34	34[23]	18	2	2	5	Veinbanding
Checks. Rubbed with juice of healthy tobacco	327	0	.....	0	....	.....	.....

[23]Infected plants showed symptoms of veinbanding only.

lations made June 16, 1928. Chlorotic, irregular to circular spots developed in 9 days on the rubbed leaves of all plants inoculated with the mosaics. A few days later, mottling and necrotic spots appeared on unrubbed leaves. The mild mosaics caused stunting, but little or no distortion, whereas the two severe mosaics and ring mosaic caused marked stunting and distortion. Yellow mosaic plants were stunted and distorted and had a

TABLE 22

Inoculations of Tomato in the Field with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco in the greenhouse.			
				Number plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	6	6	10	1	1	8	Severe mosaic type 1
Mild mosaic type 1	6	6	10	1	1	8	Mild mosaic type 1
Severe mosaic type 2	6	6	10	1	1	8	Severe mosaic type 2
Mild mosaic type 2	6	6	10	1	1	8	Mild mosaic type 2
Yellow mosaic	7	7	10	1	1	8	Yellow mosaic
Ring mosaic	7	7	10	1	1	8	Ring mosaic
Etch	6	6	10	1	1	8	Etch
Etch+	6	6	10	1	1	8	Etch+
Severe etch	5	5	10	1	1	6	Severe etch
Veinbanding	6	0	.....	1	0	.....	.....
Coarse etch	6	0	.....	1	0	.....	.....
Ringspot	6	0	.....	1	0	.....	.....
Cucumber mosaic type 1	13	(?)[24]	.....	2	0	.....	.....
Cucumber mosaic type 2	6	(?)[24]	.....	1	0	.....	.....
"Healthy potato" virus	16	16	10	1	1	28	"Healthy potato" virus
"Healthy potato" virus+ veinbanding	5	5	10	1	1	8	"Healthy potato" virus
Cucumber mosaic type 3+veinbanding	5	?	.....	1	0	.....	.....
Checks. Rubbed with juice from healthy tobacco	10	0	.....	1	0	.....	.....

[24]Plants seemed abnormal but symptoms were indefinite.

marked yellowing between the veins. Some mosaic plants produced a few small, mosaic-mottled fruits, while others produced none.

The etch viruses produced a mosaic-like mottling on field plants like that in the greenhouse. Plants in the field were distorted and stunted. Affected plants produced a few small, unmottled fruits.

Two plants of 6 inoculated with the virus of coarse etch developed a mosaic-like mottling 1 month after inoculation. No other symptoms developed and normal fruits were produced. Inoculations to tobacco from these plants resulted in typical coarse etch.

The results of the inoculations with the viruses of veinbanding, ringspot and the cucumber mosaics appeared negative, altho, with the latter, chlorotic spots appeared on rubbed leaves 12 days after inoculation.

"Healthy potato" virus and this with veinbanding produced symptoms on field plants like those on greenhouse plants inoculated with the "healthy potato" virus.

The transfers back to tobacco, which were not made until 50 days after inoculation, indicate the necessity of prompt transfers from field plants when insects are numerous, if contaminations are to be avoided. Seven etch +, 3 severe etch, and 1 severe mosaic type 1 came from plants that had not been inoculated with these viruses. Aphids, cucumber beetles, and flea-beetles were numerous on these plants. The plants had not been touched, indicating that these infections may be attributed to insects.

To cucumber. White Spine cucumber seed were planted in hills 6 feet apart each way on May 9, 1928. Soon after the plants were up they were caged with tobacco cotton and the latter was not removed until the inoculations were made on June 21, 1928. The results of these inoculations are given in Table 24.

Infections resulted in the plants inoculated with the viruses of cucumber mosaics, types 1, 2, and 3, and ringspot. The symptoms were identical with those described for these viruses on greenhouse plants. Cucumber mosaic type 3 caused greater injury than types 1 and 2, and the latter caused more injury than type 1. All the cucumber plants infected with cucumber mosaics produced typical white pickle. The transfers to tobacco resulted in the expected types. The importance of making early transfers, where insects are numerous, is again shown in the transfers to tobacco, where cucumber mosaics were obtained from 4 plants that had not been inoculated with these viruses. A

TABLE 23

Inoculations of Pepper in the Field with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco in greenhouse.			
				Number plants inoculated.[25]	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	6	6	9	2	2	7–10	Severe mosaic type 1+etch+
Mild mosaic type 1	6	6	9	2	2	7	Mild mosaic type 1
Severe mosaic type 2	6	6	9	2	2	7	Severe mosaic type 2
Mild mosaic type 2	6	6	9	2	2	7	Mild mosaic +etch+
Yellow mosaic	6	6	9	2	2	7	Yellow mosaic
Ring mosaic	6	6	9	2	2	7	Ring mosaic +etch
Etch	6	6	9	2	2	5	Etch
Etch+	6	6	9	2	2	4	Etch+
Severe etch	6	6	9	2	....	.....	.....
Veinbanding	6	?	.....	2	2	5–6	Etch+
Coarse etch	6	2	9	2	2	10 24	Coarse etch
Ringspot	6	0	.....	2	2	6	Etch+
Cucumber mosaic type 1	12	?	.....	4	4	8–10	2 Etch 1 etch+and 1 severe etch
Cucumber mosaic type 2	6	?	.....	2	1	10	Etch+
"Healthy potato" virus	18	18	7–9	6	1	8	Severe etch
"Healthy potato" virus+ veinbanding	6	6	9	2	2	5	"Healthy potato" virus+ veinbanding+ severe mosaic
Cucumber mosaic type 3+veinbanding	6	?	.....	2	2	10	Etch+
Checks. Rubbed with juice from healthy tobacco	13	0	.....	....	0	.....	

[25]Inoculations back to Turkish tobacco were made 50 days after the inoculations were made to pepper.

further reference will be made to insect transmissions of the viruses.

To muskmelon. Seed of Pollard muskmelon (*Cucumis melo*) were planted in hills 6 feet apart each way on May 9,

1928. The plants were caged with tobacco cotton soon after they were up and it was not removed until the inoculations were made on July 6, 1928. Only the cucumber mosaics gave infection on muskmelon and the leaf symptoms were identical with those produced by these viruses on cucumber. The percentages of infection were lower, however, than with the cucumber.

To Nicotiana rustica. Plants were pulled from a bed covered with tobacco cotton after the hands had been thoroly washed with soap and water, and set, on May 28, 1928. The inoculations were made July 3, 1928, after carefully examining each plant for any symptoms of virus diseases. The results of these inoculations are given in Table 25.

Six tobacco mosaics used in this test produced the same symptoms on *N. rustica.* Numerous small, necrotic spots appeared on rubbed leaves 6 days after inoculation. At this time the unfolding bud leaves were chlorotic and twisted and necrosis of the spot type continued to develop on the new leaves as rapidly as they formed. All the mosaic plants were conspicuously chlorotic, stunted, and malformed. Many of the plants were dead a month after inoculation, while those that were living at this time consisted of a few unrubbed leaves at the bases and tufts of small, twisted, chlorotic leaves near the tips where a few malformed flowers were being produced. It was impossible to distinguish one mosaic from another on this species and the symptoms would not suggest mosaic to one acquainted only with mosaic on tobacco (*N. tabacum*).

Etch and etch + plants developed a chlorotic spotting like that described for these types on tobacco. A mosaic-like mottling developed a few days later and the leaves had a slight rugose appearance. Both of these viruses caused a small amount of distortion and stunting. Plants inoculated with severe etch developed small, chlorotic spots on both rubbed and unrubbed leaves in 11 days. The new leaves had a characteristic wrinkled appearance 15 days after inoculation. The plants were dwarfed and the leaves were slightly distorted.

The early symptoms produced by coarse etch on field plants of *N. rustica* are like those caused by this type on greenhouse plants but coarse etch necrotic patterns and the chlorotic patterns in the new leaves were not observed in the field plants.

Only 1 plant of 8 inoculated with ringspot developed symptoms. The chlorotic rings were not numerous and did not become necrotic.

Inoculations with the virus of veinbanding, the cucumber mosaics, and "healthy potato," alone and with veinbanding, were not satisfactory. One plant of 18 inoculated with cucum-

TABLE 24

Inoculations of Cucumber in the Field with Tobacco Viruses.

Virus type	Number plants inoculated.	Number positive.	Days to first symptoms.	Inoculations back to Turkish tobacco in greenhouse.			
				Number plants inoculated.	Number positive.	Days to first symptoms.	Type transmitted.
Severe mosaic type 1	30	0	.....	0	....	.....	.....
Mild mosaic type 1	30	0	.....	0	....	.....	.....
Severe mosaic type 2	30	2	19	2	2	5–8	Cucumber mosaic type 1
Mild mosaic type 2	30	2	19	2	2	5	Cucumber mosaic type 2
Yellow mosaic	30	0	.....	0	....	.....	.....
Ring mosaic	18	0	.....	0	....	.....	.....
Etch	30	2	19	2	2	13	Cucumber mosaic type 2
Etch+	30	0	.....	0	....	.....	.....
Severe etch	12	0	.....	0	....	.....	.....
Veinbanding	30	0	.....	0	....	.....	.....
Coarse etch	30	0	.....	0	....	.....	.....
Ringspot	30	14	15–19	3	2	8	Ringspot
Cucumber mosaic type 1	60	24	9–23	8	8	7–13	Cucumber mosaic type 1
Cucumber mosaic type 2	30	20	7–19	6	6	8–13	Cucumber mosaic type 2
"Healthy potato" virus	60	4	2	4	4	6–13	Cucumber mosaic type 2
"Healthy potato" virus+ veinbanding	30	0	.....	0	....	.....	.....
Cucumber mosaic type 3+veinbanding	30	22	14–23	2	2	7	Cucumber mosaic type 3
Checks. Inoculated with juice from healthy tobacco	162	0	.....	0	....	.....	.....

ber mosaic type 1 developed chlorosis and slight distortion 1 month after inoculation. Since no transfers were made to tobacco, this is a doubtful case of infection. Two plants inoculated with cucumber mosaic type 2 developed chlorotic patterns like those described for this type on tobacco, a condition not observed in any other plant in this test.

The results of the inoculations with "healthy potato" virus and "healthy potato" virus + veinbanding appeared negative.

To various cultivated species. Inoculations were made with viruses to white mustard (*Brassica alba*), Early Scarlet Turnip radish (*Raphanus sativus*), Early Purple Top Strap Leaf turnip (*Brassica rapa* var. *depressa*), First and Best peas (*Pisum sativum*), Stringless Greenpod bean (*Phascolus vulgaris*), Crosby's Egyptian beet (*Beta vulgaris*), American Purple Top rutabaga (*Brassica oleracea*), and Virginia soybean (*Soja max*).

The only species that showed any evidence of infection in these inoculations was the beet, altho all plants were grown to maturity. Beet inoculated with the cucumber mosaics, alone or in combination with veinbanding, developed necrotic rings ⅛ inch in diameter on rubbed leaves 10 days after inoculation. These rings increased in size to ¼ inch in diameter, at which time they were composed of alternate bands of necrotic and red tissues. No other symptoms developed.

"Healthy potato" virus produced necrotic rings 1/16 inch in diameter on rubbed leaves 10 days after inoculation. These rings did not increase in diameter like those produced by the cucumber mosaics. "Healthy potato" virus + veinbanding plants had the same symptoms as those inoculated with "healthy potato" virus alone.

These inoculations indicate that, under the conditions of the experiment, the species tested are not susceptible to the virus diseases, because the same method of inoculation under field conditions gave infection on susceptible plants such as tobacco, tomato, pepper, and *N. rustica*.

INSECT TRANSMISSION OF VIRUSES

Observations made in the field indicate that insects may be responsible for the spread of virus diseases in tobacco and other

TABLE 25

Inoculations of Nicotiana Rustica in the Field with Tobacco Viruses.

Virus type	Number plants inoculated	Number positive	Days to first symptoms
Severe mosaic type 1	10	10	6
Mild mosaic type 1	9	9	6
Severe mosaic type 2	9	9	6
Mild mosaic type 2	9	9	6
Yellow mosaic	9	9	6
Ring mosaic	9	9	6
Etch	10	10	20
Etch+	10	10	20
Severe etch	8	8	11–17
Veinbanding	11	0	.....
Coarse etch	9	1	20
Ringspot	8	1	6
Cucumber mosaic type 1	18	1 (?)	.....
Cucumber mosaic type 2	10	2	11
"Healthy potato" virus	24	0	.....
"Healthy potato" virus+vein-banding	8	0	.....
Cucumber mosaic type 3+vein-banding	9	0	.....
Checks. Rubbed with juice from healthy tobacco	49	4	2 severe etch, 1 mosaic and 1 ringspot

plants. The experiments reported in Tables 21 to 25, inclusive, were made in an area of about ¼ acre. Two species of cucumber beetle, the striped (*Diabrotica vittata*), and the spotted (*D. duodecem-punctata*), fleabeetles, and leafhoppers were numerous on cucumber, muskmelon, tomato, pepper, *N. rustica,* and tobacco, in spite of frequent dustings of all the plants (except tobacco) with calcium arsenate and gypsum. Aphids were first observed on tomato and pepper June 25, 1928, but were not seen on tobacco. As was pointed out in the description of these experiments, every precaution was taken to prevent infection by handling. The plants were cultivated by a man who was instructed not to touch the plants, and the cultivator was run midway between the rows so that there was little likelihood of spreading the viruses by this means.

Inspections were made of the cucumber plants on July 13, when 41 cases of cucumber mosaic and 5 of ringspot were

observed on plants that had not been inoculated with these viruses.

By August 6, nearly all the pepper plants were affected with virus diseases, and inoculations made from some of them to tobacco indicated that there had been considerable spread of at least one virus (etch +). See Table 23.

Inspections were made of the tobacco on September 13, before topping and suckering. Eighty-one tobacco mosaics, 21 etch, 102 etch +, 12 severe etch, and 12 veinbanding plants were found either in checks or in plants that had not been inoculated with the virus of the disease found. This again suggests insect transmission.

Further evidence of insect transmission is suggested in the examination of the record of Farm 22a, Table 9. The record shows the presence of 23.81% etch, 7.04% veinbanding, 1.9% ringspot, and no tobacco mosaic. As this observation was made before topping and suckering, and as none of the viruses found survive drying, it is probable that they came from weed hosts and were transmitted from these to the tobacco by insects.

The observations in Table 8 show that the percentages of virus diseases gradually increase as the season advances. All the observations except the fourth were made prior to any of the handling operations necessary in caring for the tobacco crop. As solanaceous weeds are numerous on the Experiment Station farm and as many of them are infected, it is probable that many of these viruses were transmitted to the tobacco from infected weeds by insects.

GROUPING OF VIRUSES

The studies indicate that the virus diseases that affect tobacco may be divided into 7 groups on a basis of the symptoms which they produce on tobacco and other hosts, host range and reaction to drying. The first group includes the true tobacco mosaics, which apparently affect only the solanaceae and which retain their infectivities after long periods of drying. The other groups are: the etch viruses, cucumber mosaics, coarse etch, veinbanding, ringspot, and "healthy potato" virus. None of the viruses in these last 6 groups survives in air-dried tissue, but some of them affect plants belonging to other fam-

ilies besides the solanaceae. These, so far as present evidence indicates, are ringspot, the cucumber mosaics, "healthy potato" virus and coarse etch. The following brief descriptions of symptoms produced by the viruses on various hosts are given in the hope that they may be an aid to others in identifying some of the virus diseases that affect tobacco, tomato, pepper, cucumber, muskmelon and some solanaceous weeds.

KEY TO VIRUSES

I. Infectivity not destroyed by air drying.

A. Infection on solanaceae only.

1. True tobacco mosaics.

On tomato:

Mottling inconspicuous to marked; distortion none to medium; yellow tissue between the veins (yellow mosaic); chlorotic and necrotic rings on leaves and fruit and necrotic streaks on stem and petioles (ring mosaic). White tissue between the veins, irregular white areas on fruit (white mosaic).

On pepper:

Mottling on leaves and sometimes on fruit; distortion slight to marked; stunting; necrosis; yellow tissue between the veins (yellow mosaic); concentric yellow rings on leaves (ring mosaic); yellow to almost white areas between the veins (white mosaic).

On *Physalis heterophylla*:

Chlorotic spots on rubbed and occasionally on unrubbed leaves; no distortion; no mottling.

On *Physalis pubescens*:

Chlorotic spots on rubbed and unrubbed leaves; mosaic-like mottling; distortion none to slight; yellow areas between the veins (yellow mosaic); yellowing, distortion, stunting (white mosaic).

On horsenettle:

Mottling faint to medium; distortion none to medium; stunting none to medium; necrosis (ring mosaic).

On jimson:

Local necrosis; necrotic stem streaks; apparently not systemic.

On eggplant:

Necrotic spots on rubbed leaves; necrosis of stems and petioles; no mottling; probably not systemic.

On *Nicotiana rustica*:

Marked necrosis; chlorosis; distortion; stunting; sometimes death in the field.

On apple of Peru:

Chlorotic spots with green borders on rubbed leaves; necrosis sometimes present around spots.

On tobacco:

Typical mottling; necrosis little to marked; little or no distortion or stunting (mild mosaics); distortion medium to marked (severe mosaics); yellow tissue between the veins (yellow mosaic); chlorotic ring-like patterns (ring mosaic); yellow to almost white tissue between the veins (white mosaic).

On cucumber:

No infection.

On muskmelon.

No infection.

II. Infectivity destroyed by drying.

A. No infection on cucumber.

1. Etch viruses (etch, etch +, severe etch).

On tomato:

Mottling; distortion little to marked; stunting.

On pepper:

Leaf mottling; stunting; distortion.

On *P. heterophylla*:
Mosaic-like mottling; stunting; distortion.

On *P. pubescens*:
Vein clearing; leaf surface warty; distortion; stunting.

On horsenettle:
Irregular chlorotic patterns along veins; no mottling; slight distortion.

On jimson:
Vein clearing; mottling; distortion; leaves narrow, often malformed and string-like; stunting.

On eggplant:
No infection.

On *N. rustica*:
Spotting; mottling; distortion; stunting; wrinkling (severe etch).

On apple of Peru:
Mottling; stunting; distortion; speck-necrosis.

On tobacco:
Tiny, necrotic rings and arcs of circles on rubbed leaves; fine, necrotic etching; circular chlorotic spots in growing point leaves; stunting; ochre yellow to brown necrotic veins in older leaves of field plants; distortion, rugoseness, alligator hide effect (etch +); marked stunting, distortion, wrinkling, necrosis of both midrib and veins in field plants (severe etch.)

On cucumber:
No infection.

On pokeweed:
No infection.

2. Veinbanding.

On tomato:
Faint chlorosis in vein islets.

On pepper:
Indefinite symptoms.
On *P. heterophylla*:
Indefinite symptoms.
On *P. pubescens*:
Small chlorotic spots along veins.
On horsenettle:
No infection (?)
On jimson:
No symptoms; infection (?)
On eggplant:
No symptoms; infection.
On *N. rustica*:
Chlorotic spots; dark tissue along veins.
On apple of Peru:
No infection.
On tobacco:
Vein chlorosis; faint chlorotic spots in new leaves; narrow bands of dark green tissue along veins of older leaves; drooping of lower leaves.
On cucumber:
No infection.
On pokeweed:
No infection.

3. Coarse etch.
On tomato:
Infection (?)
On pepper:
No infection.
On *P. heterophylla*:
Coarse etch chlorotic and necrotic patterns in new leaves.
On *P. pubescens*:
Indefinite chlorotic curved line patterns in new growth.
On horsenettle:
No infection.

On jimson:
Mottling.
On eggplant:
Mottling; chlorotic patterns along veins.
On *N. rustica*:
Symptoms as for this type on tobacco.
On apple of Peru:
Small necrotic spots on leaves and calyx tubes.
On tobacco:
Irregular to ring-like patterns with narrow necrotic circumferences; chlorotic ring and line patterns in new leaves; no distortion; no stunting; symptoms nearly always present in growing point.
On cucumber:
No infection.
On pokeweed:
Local coarse etch necrotic patterns along veins of rubbed leaves.

4. "Healthy potato" virus.
On tomato:
Faint mottling; chlorotic spots; necrotic speck-spots in the field.
On pepper:
Faint chlorotic and necrotic spots.
On *P. heterophylla*:
Faint mottling; irregular chlorotic patterns along the veins.
On *P. pubescens*:
Small, chlorotic spots between the veins.
On horsenettle:
Faint mottling.
On jimson:
Small necrotic rings; faint mottling.
On eggplant:
No symptoms; infection.
On *N. rustica*:
Symptoms as on tobacco.

On tobacco:

Small, necrotic circles usually with a pinpoint necrotic center on rubbed leaves; watered silk chlorotic and necrotic line patterns in new growth.

On beet:

Small, chlorotic and necrotic rings on rubbed leaves.

On cucumber:

No infection.

On pokeweed:

Small, chlorotic circles on rubbed leaves.

B. Infection on cucumber.

1. Cucumber mosaics (types 1, 2 and 3).

On tomato:

Mottling; distortion; stunting; leaves often fern-leaf-like; spindling growth.

On pepper:

Pale green to yellow concentric rings on rubbed and unrubbed leaves; distortion none to medium.

On *P. heterophylla*:

Mosaic-like mottling; distortion; stunting.

On *P. pubescens*:

Chlorotic patterns along veins; distortion none to slight; stunting.

On horsenettle:

Obscure chlorotic spots; distortion (type 3).

On jimson:

Chlorotic spots on rubbed leaves.

On eggplant:

Mottling; necrosis; slight distortion.

On *N. rustica*:

Chlorosis; distortion; puffing; stunting.

On apple of Peru:

Chlorotic spots on rubbed and unrubbed leaves; necrotic spots on leaves; necrotic streaks on stem; stunting.

On tobacco:

Chlorosis; no distortion or stunting, yellow spots on rubbed leaves (type 2); medium distortion, stunting, necrosis and puffing (type 1); marked distortion, stunting, puffing and necrosis (type 3).

On cucumber:

Mottling; distortion; stunting; chlorosis; necrosis; white pickle.

On pokeweed:

Chlorotic spots on rubbed leaves; faint mottling; distortion; necrosis; stunting.

On beet:

Necrotic rings on rubbed leaves.

2. Ringspot.

On tomato:

No infection.

On pepper:

No infection.

On *P. heterophylla*:

Rings (?)

On *P. pubescens*:

No infection.

On horsenettle

Rings (?)

On jimson:

Necrotic rings; stunting; severe injury.

On eggplant:

No infection (?)

On *N. rustica*:

Chlorotic and necrotic rings.

On apple of Peru:

Chlorotic and necrotic rings.

On tobacco:

Chlorotic and necrotic rings of variable sizes; necrotic border, usually wide; oak-leaf patterns; browned and pinched tips, especially on sucker growth; growing point leaves tend to become healthy in appearance after a time.

On cucumber:

Chlorotic spots and chlorotic and necrotic rings on leaves; chlorotic rings on fruit; stunting slight to medium; no white pickle.

On pokeweed:

Numerous tiny chlorotic and necrotic rings on rubbed and unrubbed leaves.

DISCUSSION

The evidence for the existence of a number of different virus diseases affecting tobacco is based on the symptoms produced on tobacco and other hosts. Objections to basing descriptions and names upon symptomology have been advanced, but in the present imperfect state of our knowledge of the nature of the causal agent, these objections do not seem valid. Symptoms as distinct as they are in tobacco, that remain relatively constant in numerous transfers thru tobacco and other hosts under the changing conditions in the field and greenhouse, offer a fairly reliable basis for distinguishing types until a more accurate one is devised.

It cannot be said definitely that the virus diseases described in this bulletin are not due to mixtures, because mixtures of viruses in tobacco and potato are not uncommon. Tests indicate that white mosaic is a mixture of a green and a white with either a predominance or a greater virulence of the latter. It is possible that a complete separation might be effected by serial needle transfers thru tobacco with inoculum taken from white and green areas, respectively. Yellow mosaic and even ring mosaic may be mixtures, and serial needle transfers should be made. It is doubtful whether separations of mixed tobacco mosaics can be effected by dilutions because all of them probably retain their infectivities at or near the same high dilutions. Tobacco affected with the green mosaics (severe and mild) usually has a few small yellow spots, indicating that there may be a mixture. These yellow spots are usually more numerous in tobacco that has been inoculated from naturally-infected horsenettle and groundcherry. If mixtures occurred in the other viruses, they were of types or strains so nearly alike that they were not detected by the technic used in these experiments.

There has been no evidence of permanent attenuation of any of the viruses in these tests. It might be suggested that the mild mosaics are attenuated forms of the severe mosaics and that etch is an attenuated form of etch + or severe etch. If this were true, severe mosaics should, under certain conditions, develop symptoms of mild mosaic, and severe etch and etch + should, at some time, show the less prominent symptoms of etch. Changes of this kind have not been observed at any time. If the viruses are living entities, it is not difficult to believe that there may be strains of these just as there are of bacteria and fungi. Mixtures of strains, when the plants are subjected to variable conditions, might cause atypical symptoms due to the elimination of one or more strains or to the non-expression of symptoms by one or more strains. This might be mistaken for evidence of attenuation.

The use of a number of hosts in virus studies is valuable in establishing the identity of an unknown virus disease on such plants as tomato, pepper and solanaceous weeds. For example, among the virus diseases affecting tomato are tobacco mosaics, cucumber mosaics, and the etch viruses, the symptoms of which

are similar enough on tomato to be confused, especially in the field. Inoculations to tobacco from virus-diseased tomato plants usually result in symptoms that are readily recognized as characteristic for some known virus disease. If there should still be doubt as to the identity of the virus disease concerned, further transfers to cucumber and jimson may be made. If infection results on cucumber, it indicates the presence of a cucumber mosaic, whereas if no symptoms occur on cucumber, the virus may be either a tobacco mosaic or an etch virus. If jimson plants develop necrotic stem streaks, a tobacco mosaic is indicated, whereas if jimson develops vein clearing, mottling, and malformation, an etch virus is indicated. Another example may be cited. Cucumber is susceptible to ringspot and cucumber mosaics, the symptoms of which might be confused in field-affected plants. If infection develops on tomato from inoculations made from cucumber, cucumber mosaic is indicated, because tomato appears not to be susceptible to ringspot. Tobacco is susceptible to both ringspot and cucumber mosaics, and the symptoms of each are distinct.

Inoculations to more than 1 host may be of value in separating virus mixtures that may occur in the same plant. This may be illustrated by the mixture of cucumber mosaic type 3 and veinbanding. Cucumber is not susceptible to veinbanding, and inoculations to this species give only cucumber mosaic type 3, when transferred back to tobacco. Etch viruses are sometimes mixed with tobacco mosaics. A separation may be effected by inoculating jimson with the mixture. The tobacco mosaic causes only local necrosis, and if non-necrotic leaves are used as inoculum in subsequent transfers to tobacco, etch only should be obtained.

There is no indication in these studies that the properties of the viruses are dependent upon the host which they infect, as Walker (35) suggested in his studies of cucumber, tomato, and Physalis mosaics. It is conceivable for one to have a mixture of cucumber and tomato mosaic (tobacco) and not be able to distinguish between the symptoms produced by these 2 viruses on tomato and possibly on Physalis.

Of the virus diseases described in this paper, severe and mild mosaics are present in most tobacco fields in Kentucky.

Yellow and ring mosaics are rarely found, and it is not certain that white mosaic occurs as a distinct disease of tobacco in Kentucky. Etch, etch +, and veinbanding occur usually only in areas where vegetables, especially potatoes, are or have been grown and where solanaceous weeds are present. The presence of ringspot and coarse etch in most fields suggests that these may have a wider host range than some of the other viruses.

The effect that the presence of virus diseases may have on the yield and quality of tobacco has not been investigated except for tobacco mosaics. Valleau and Johnson (30) and McMurtrey (18) have shown that tobacco mosaic decreases the yield and lowers the quality of tobacco. It is almost certain that early and extensive infections of ringspot, etch viruses and cucumber mosaics have an adverse effect on the yield and quality of tobacco. It is doubtful whether damage is caused by these viruses when infection occurs in plants that are approaching maturity. The percentage of coarse etch is never large, and usually only a few leaves of a plant are affected; therefore, it is unlikely that this disease will be of economic importance in tobacco. Veinbanding, altho frequently present in a relatively large number of plants in some areas, does not seem to affect tobacco adversely. Some observations indicate that veinbanding plants mature earlier than healthy ones, which may be an advantage in some seasons.

Tomato and pepper are affected in nature not only with tobacco mosaics but with the etch viruses and cucumber mosaics, which may cause as great or greater injury than the former. Cucumber and other cucurbits are affected with ringspot and cucumber mosaic, both of which, especially the latter, may reduce yields. Since all these viruses are present in naturally-infected solanaceous weeds, their eradication is of importance in areas where these crops are grown. Gardner and Kendrick (10) have shown the importance of weed eradication in controlling "tomato mosaic," and Doolittle and Walker (7) made similar recommendations for the control of cucumber mosaic. There is an indication that some of the tobacco viruses (etch viruses, veinbanding and ringspot) are of importance as causes of potato diseases. The facts suggest the importance of

eradicating solanaceous weeds in areas where potatoes are grown for certification (33).

These studies indicate the importance of determining the identity of a virus disease on such plants as tomato, pepper and solanaceous weeds before quantitative experiments are made or conclusions drawn.

SUMMARY

Results of inoculations to Turkish tobacco in the greenhouse from naturally-infected tobacco in the field indicate the existence of a number of apprently different types of virus diseases. The symptoms produced on tobacco (Burley and Turkish) by severe tobacco mosaics types 1 and 2, mild types 1 and 2, yellow, white, and ring mosaics, etch, etch +, severe etch, veinbanding, coarse etch, ringspot, "healthy potato" virus, cucumber mosaics types 1, 2 and 3, "healthy potato" virus + veinbanding ("spot necrosis"), and cucumber mosaic type 3 + veinbanding are described. Symptoms are described also for the viruses, wherever infection developed, on tomato, pepper, *Physalis heterophylla*, *P. pubescens*, horsenettle, jimson, cucumber, muskmelon, pokeweed, eggplant, *Nicotiana rustica*, and apple of Peru.

It is pointed out that there has been no observable change in the properties of the viruses by reason of successive transfers thru tobacco, and no changes in the viruses have been noted by reason of their sojourn in other susceptible species.

Two diseases of tobacco, each caused by a mixture of 2 viruses, and each originally thought to be caused by a single virus, were studied. These were later found to be diseases due to mixed viruses, and the changes observed in symptoms were found to be due to the loss of 1 virus of the mixture.

The possibility that many viruses may over-winter in perennial solanaceous weeds is indicated by the results of inoculating tobacco with virus from naturally-infected species of this family. Eight virus types: severe mosaic type 1, mild mosaic type 1, yellow mosaic, etch, etch +, severe etch, ringspot, and cucumber mosaic type 1 were obtained from naturally-infected solanaceous weeds.

Inoculations of tobacco with virus from naturally-infected

tomato, pepper, cucumber, muskmelon, and petunia indicate that viruses other than tobacco mosaics are not uncommon in these species. Types obtained from these species were:

Tomato: Severe mosaic type 1, mild mosaic type 1, etch, etch +, severe etch, coarse etch, veinbanding, and "healthy potato" virus.

Pepper: Severe mosaic type 1, white mosaic, cucumber mosaic type 1, etch, etch +, and severe etch.

Cucumber and Muskmelon: Cucumber mosaic type 1 and ringspot.

Petunia: Mild mosaic type 1, etch, etch +, severe etch, cucumber mosaic type 1, and "healthy" potato" virus + veinbanding.

It is pointed out that it is difficult to identify, by visual inspection, viruses which affect tomato and pepper in nature, and the suggestion is made that diagnosis of the virus concerned should be based on transfers to tobacco, where the symptoms usually are easily recognized.

The inoculations of pokeweed with the viruses and transfers from naturally-infected pokeweed to various species susceptible to cucumber mosaic indicate that the virus disease of common occurrence in this species in Kentucky is not cucumber mosaic, or any of the other viruses studied. Cucumber mosaics, coarse etch and ringspot produce symptoms on pokeweed.

Seven apparently distinct groups of virus diseases are indicated on a basis of the symptoms which they produce on tobacco and other hosts. These groups are: tobacco mosaics, etch viruses, cucumber mosaics, coarse etch, veinbanding, ring spot, and "healthy potato" virus.

A key is given as an aid to others in identifying virus diseases that affect tobacco, tomato, pepper, cucumber, muskmelon and solanaceous weeds.

A 3-years' survey of the tobacco fields on the Experiment Station farm at Lexington and on several other farms in Fayette County indicated that the viruses found in Burley tobacco are severe mosaics, mild mosaics, etch, etch +, veinbanding, coarse etch, ringspot, and cucumber mosaic type 1. Greater percentages of some of these viruses are shown to be

present in tobacco plants growing in vegetable gardening and potato-growing areas.

Observational evidence indicates that the viruses may be transmitted by insects, and 2 species of cucumber beetle (the spotted and the striped), fleabeetles, leafhoppers and aphids are suggested as probable insect vectors.

Inoculations indicate that white mustard, radish, turnip, pea, bean, rutabaga, kale, and soybeans are not susceptible to any of the viruses under the conditions of the experiments. Cucumber mosaics and "healthy potato" virus cause local infections on beet leaves.

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PLATE I.

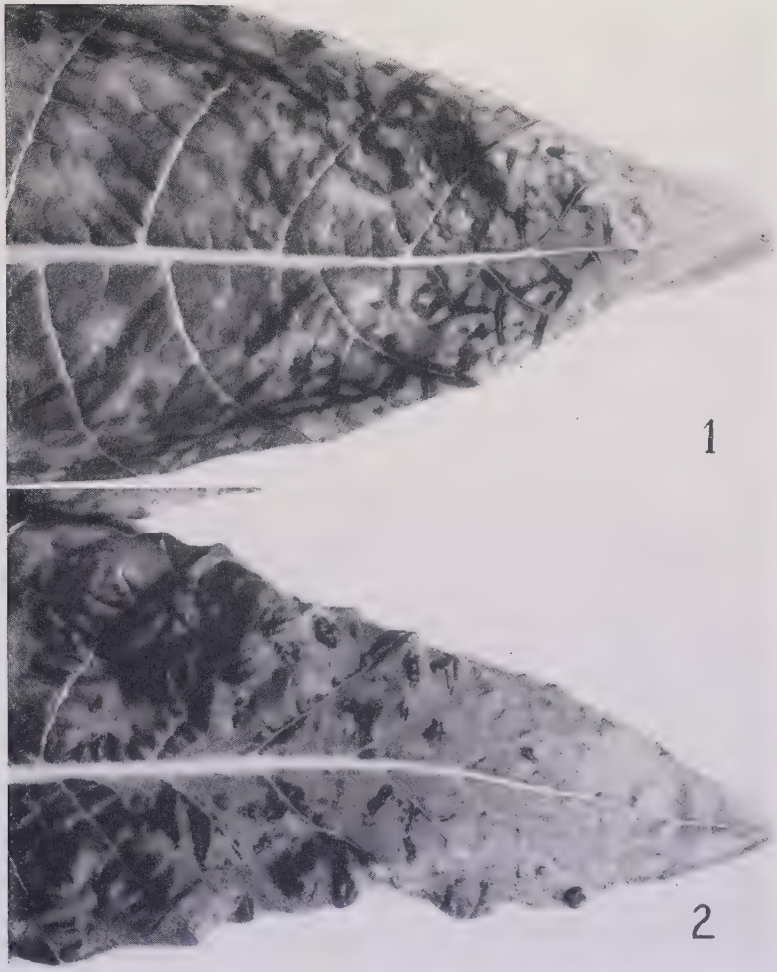


FIGURE 1. Severe mosaic type 1. Leaf from a Burley plant from a place near the tip. Plant inoculated in the field July 26, 1928. Photographed August 17, 1928.

FIGURE 2. Severe mosaic type 2. Growing point leaf comparable to that in figure 1. Plant inoculated July 26, 1928. Photographed August 17, 1928. Compare the amount of distortion with that in figure 1.

PLATE II.

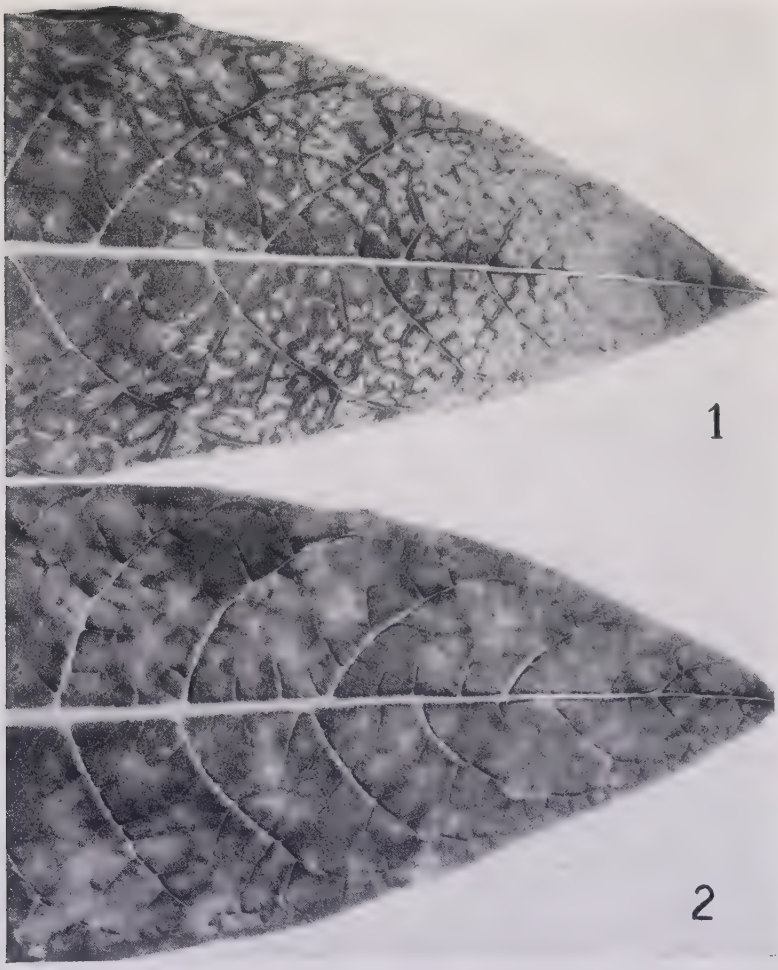


FIGURE 1. Mild mosaic type 1. Leaf from the growing point of a Burley plant inoculated in the field July 26, 1928. Photographed August 17, 1928. Compare figures 1 and 2, this plate.

FIGURE 2. Mild mosaic type 2. Leaf from the growing point of a Burley plant inoculated in the field July 26, 1928. Photographed August 17, 1928. Observe the diffused mottling.

PLATE III.

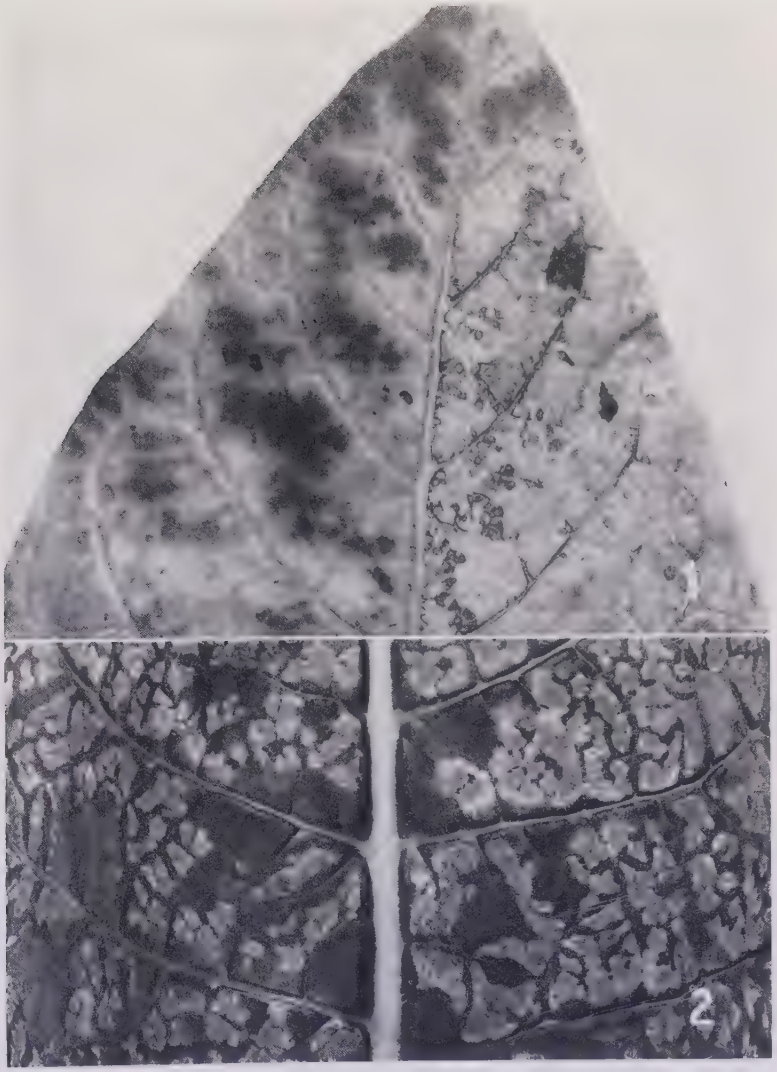


FIGURE 1. Yellow mosaic. Leaf from a Burley plant inoculated in the field July 26, 1928. This leaf shows the general chlorosis and ring-like areas of alternating bands of green and yellow tissue. The rings are prominent in the upper right half of the leaf.

FIGURE 2. Yellow mosaic. Leaf from the growing point of the plant from which the leaf in figure 1 was taken. Photographed August 15, 1928. This leaf shows the typical lemon-yellow tissue between the veins.

PLATE IV.

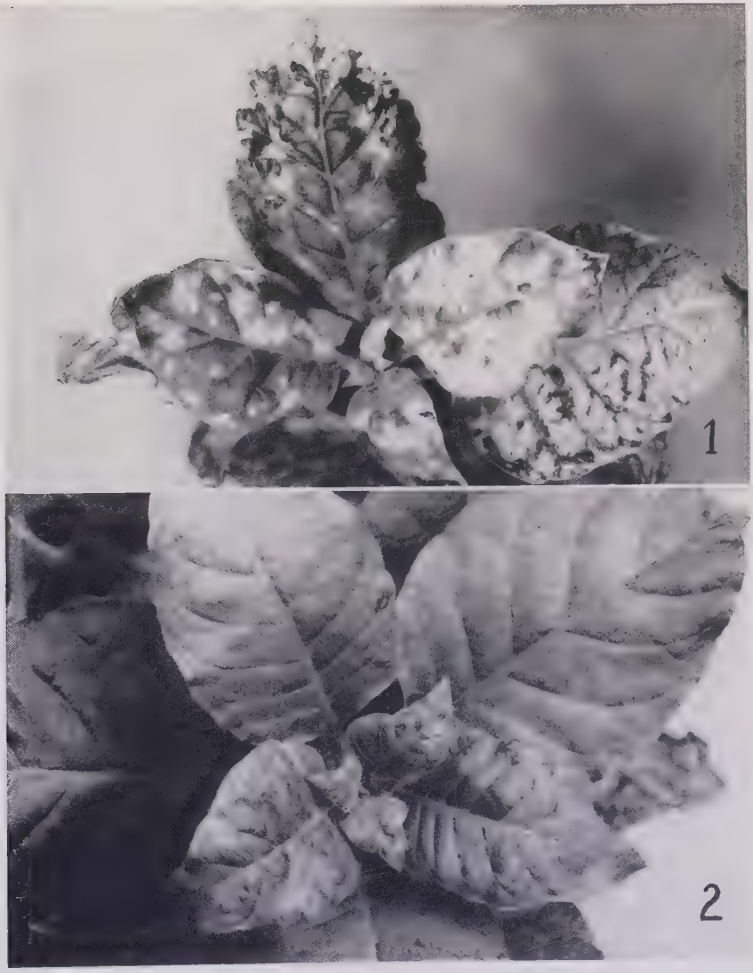


FIGURE 1. White mosaic on Turkish tobacco in the greenhouse. Plant inoculated June 12, 1929. Photographed July 7, 1929. The creamy-white and dark green blister-like areas are shown.

FIGURE 2. Severe mosaic type 2. This plant was inoculated on the same date as the plant in figure 1. The photograph was taken on the same date and at the same focal distance so that the size difference is real.

PLATE V.

FIGURE 1. Ring mosaic. Leaves from the tip of a Burley plant inoculated in the field July 26, 1928. Photographed August 15, 1928. These leaves show the ring-like spots before and after the development of necrosis. Dark tissue along the veins is shown in the upper half of the leaf, on the left.

FIGURE 2. Ring mosaic. Leaf taken near the middle of the plant from which the leaf in figure 1 came. This leaf shows both early and late stages of the spotting.

PLATE VI.

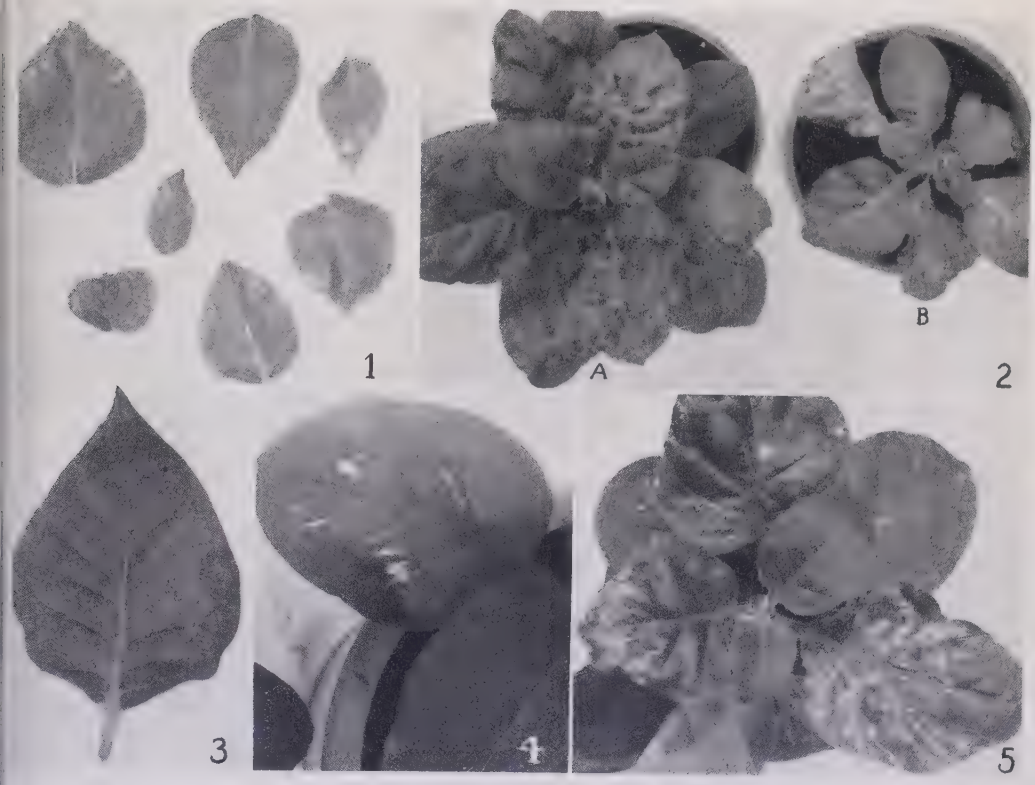


FIGURE 1. Ringspot on Cobbler potato leaves. Natural infection.

FIGURE 2. A. Etch on Turkish tobacco. Spotting on 2 upper leaves and etching on those beneath. B. Severe etch on Turkish. Distortion and stunting are shown. Same age as A.

FIGURE 3. Etch on a growing point leaf of Turkish tobacco. The chlorotic spots are typical.

FIGURE 4. Etch on Turkish. Etching visible on a rubbed and on an unrubbed leaf.

FIGURE 5. Turkish tobacco inoculated with ringspot cobbler foliage extract. Tobacco ringspot symptoms are shown. Symptoms of "healthy potato" are obscured.

PLATE VII.

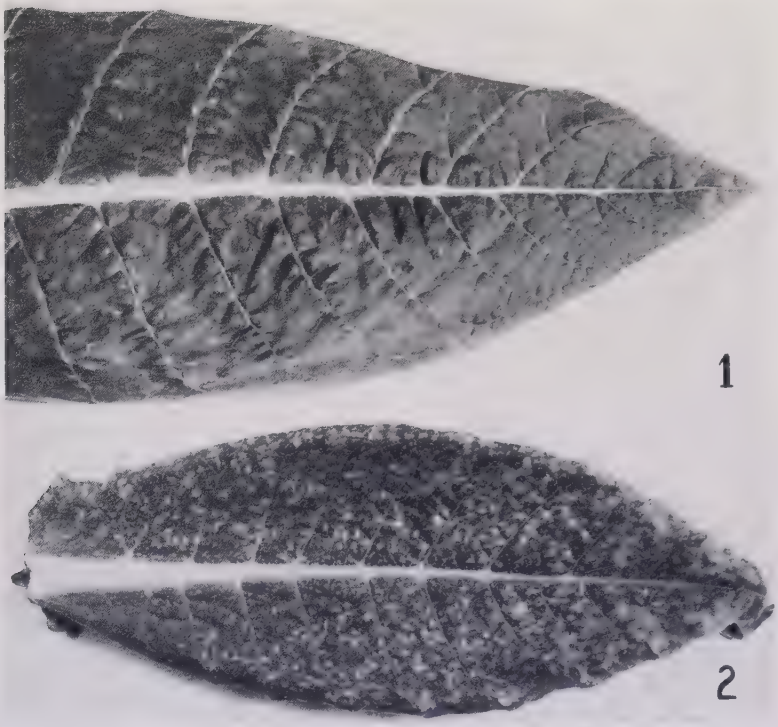


FIGURE 1. Etch+. Tip leaf from a Burley plant inoculated in the field July 26, 1928. Photographed August 17, 1928. This leaf shows the "alligator hide" effect described in the text.

FIGURE 2. Severe etch. Tip leaf from a Burley plant inoculated in the field July 26, 1928. Photographed August 17, 1928. Spotting and the inrolled margin are shown.

PLATE VIII.

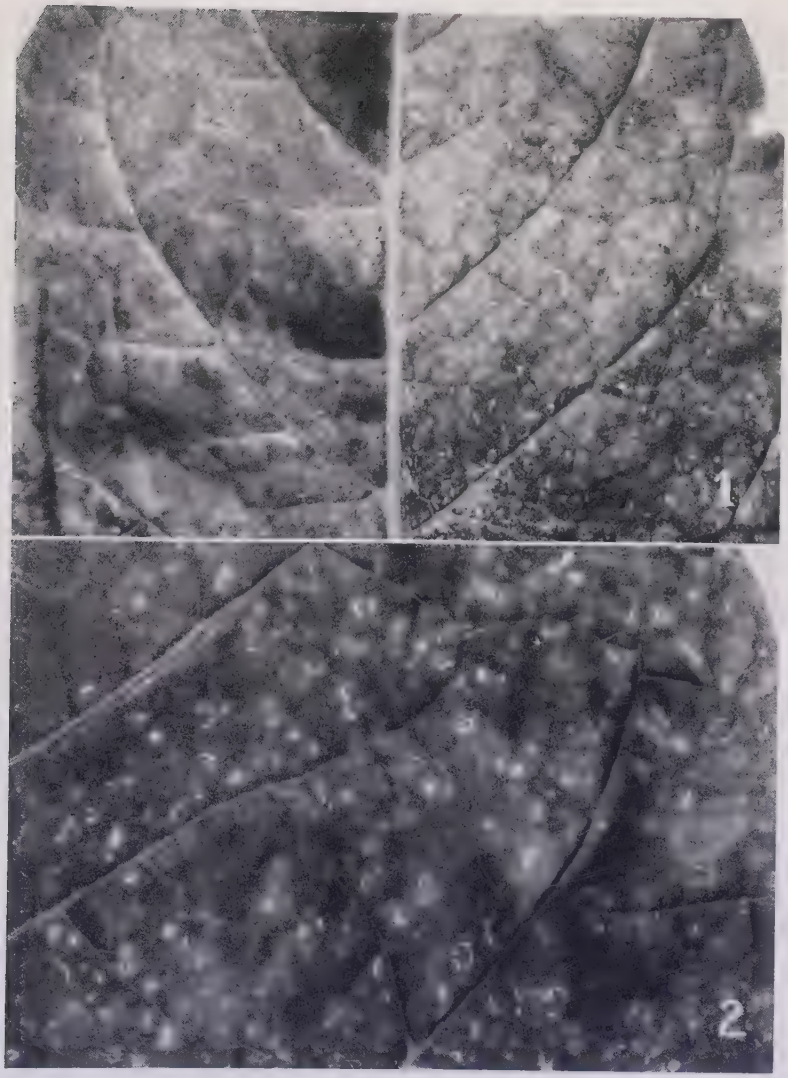


FIGURE 1. Etch on Burley tobacco. Plant inoculated in the field on July 26, 1928. Photographed August 15, 1928. Etching is shown in the right lower half and vein necrosis in the upper half of the photograph. The general chlorosis is typical.

FIGURE 2. Coarse etch on a Burley plant. Inoculated in the field July 26, 1928. Photographed August 15, 1928. Various stages of the development of necrosis are shown.

PLATE IX.

FIGURE 1. Severe etch on Burley. Plant inoculated in the field July 26, 1928. Photographed August 15, 1928. Ring-like areas with rusty-brown margins, chlorosis and necrosis are shown.

FIGURE 2. Severe etch on a lower leaf of the plant from which the leaf in figure 1 was taken. The rusty-brown necrotic veins and necrosis on the midrib are shown. Necrotic veins like these occur on field plants affected with etch and etch+but they are less prominent in the latter.

PLATE X.

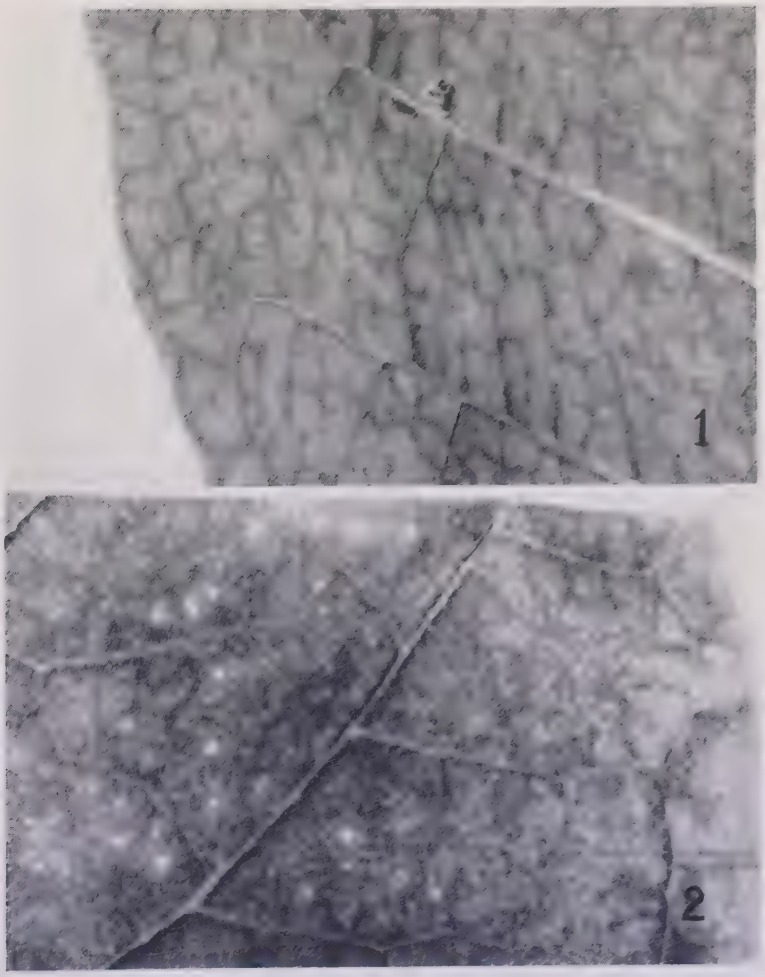


FIGURE 1. Veinbanding. Portion of a Burley leaf from a plant that was inoculated in the field July 26, 1928. Photographed August 17, 1928. The dark bands of tissue along the veins are used as the main diagnostic symptom in the field.

FIGURE 2. Coarse etch. Portion of a tip leaf of a Burley plant inoculated in the field July 26, 1928. Photographed August 17, 1928. The chlorotic ring-like and line patterns with green tissue in the centers occur both in Turkish tobacco plants in the greenhouse and in Burley plants in the field.

PLATE XI

Ringspot on Burley. Inoculation made on the first day of June, 1932. Photographed August 11, 1932. Small plants and typical leaf pattern are shown. Notice the wide variation in symptoms in comparison with the common one in green plants.

PLATE XII.

FIGURE 1. Cucumber mosaic type 1. Leaf from a naturally infected Burley plant. Photographed August 15, 1928. This type of chlorosis and malformation are typical field symptoms.

FIGURE 2. Cucumber mosaic type 1. Tip leaf from a naturally infected Burley plant. Photographed August 17, 1928. The chlorosis and puffing are shown.

PLATE XIII

Cucumber mosaic type 2. Tip leaf from a Burley plant inoculated in the field July 26, 1928. Photographed August 17, 1928. The mottling might be confused with that of tobacco mosaic but the light-colored areas are almost yellow and form more definite patterns than in tobacco mosaics.

PLATE XIV.

FIGURE 1. Ring mosaic on greenhouse-grown Turkish tobacco. The yellow rings described in the text are visible in the lower leaves. Distortion and mottling of the bud leaves, also, are shown.

FIGURE 2. Cucumber mosaic type 3+veinbanding in greenhouse Turkish tobacco. Malformation typical of these mixed viruses is shown.

FIGURE 1. Healthy June Pink tomato plants of the same age as those illustrated in figures 2 and 3 and in Plates XVI and XVII.

FIGURE 2. June Pink tomato plants. Plant on left inoculated with severe mosaic type 2 and that on right with severe mosaic type 1, on December 19, 1927. Photographed January 27, 1928. Mottling and distortion typical of these types are visible in the new growth.

FIGURE 3. June Pink tomato. Both plants inoculated with cucumber mosaic type 1 on December 19, 1927. Photographed January 27, 1928. Only the one on the left became infected. The dwarfing and string leaves are shown.

FIGURE 1. June Pink tomato. Plants inoculated with ring mosaic on January 11, 1928. Photographed January 27, 1928, to show the severe symptoms of this type. Plants in figure 1 had been inoculated 16 days when photographed while those in figure 2 had been inoculated 31 days.

FIGURE 2. June Pink tomato. Both plants inoculated with ring mosaic on December 19, 1927. Photographed January 19, 1928. The loss of the lower leaves, distortion of the new growth and stunting are typical.

FIGURE 3. Portion of a tomato fruit from a ring mosaic plant. A chlorotic ring is shown.

FIGURE 1. June Pink tomato. Both plants inoculated with etch on December 19, 1927. Photographed January 27, 1928. Compare with figure 2 this Plate and with healthy plants.

FIGURE 2. June Pink tomato. Both plants inoculated with etch+on December 19, 1927. Photographed January 27, 1928. The spindling growth and narrow leaves are typical of this virus on tomato.

FIGURE 3. June Pink tomato. Both plants inoculated with cucumber mosaic type 3+veinbanding on December 19, 1927. Photographed January 27, 1928. The dwarfing and distortion of the leaves are typical of these mixed viruses on the tomato.

PLATE XVIII.

FIGURE 1. Etch+ on Stone tomato. Plant inoculated in the field on June 15, 1928. Photographed August 18, 1928. The distortion shown here might be confused with that of tobacco mosaic.

FIGURE 2. Severe etch on Stone tomato. Plant inoculated in the field June 15, 1928. Photographed August 18, 1928. This type of distortion is typical of this virus on tomato. Severe etch produces more prominent mottling and distortion than any of the tobacco mosaics.

PLATE XIX.

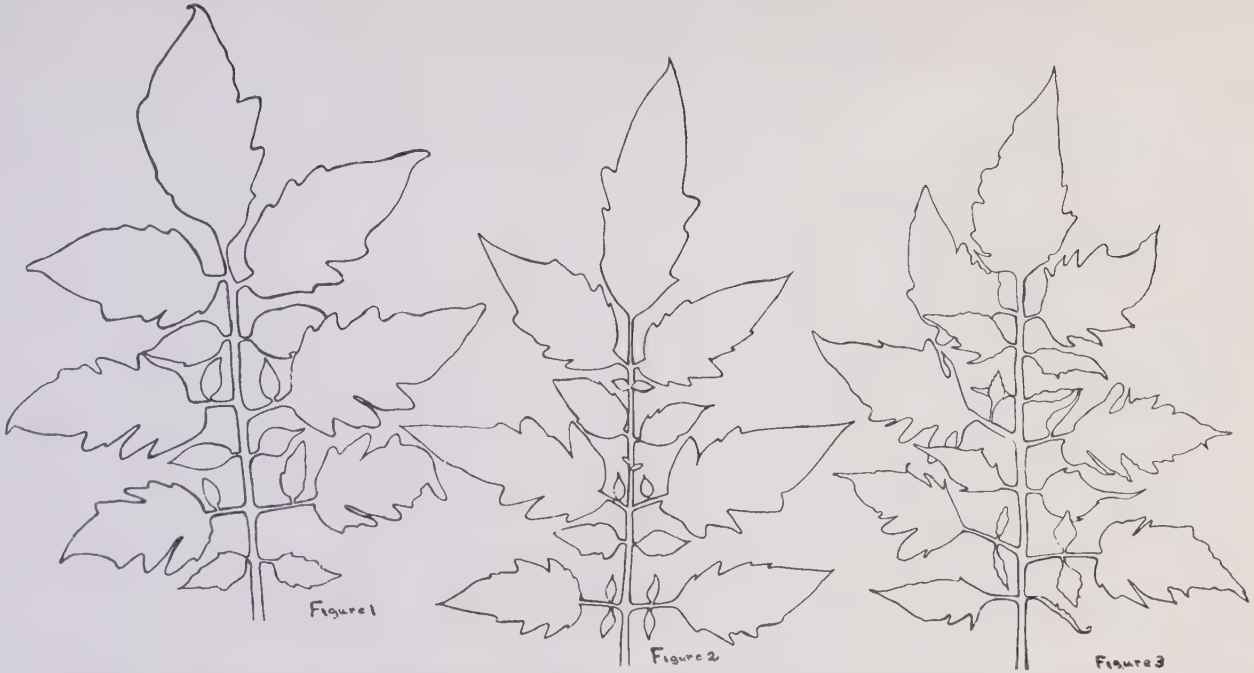
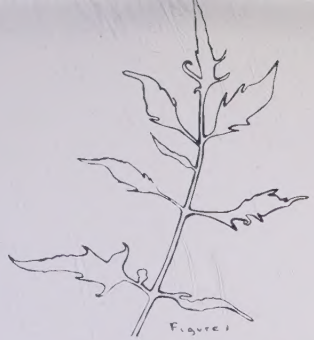
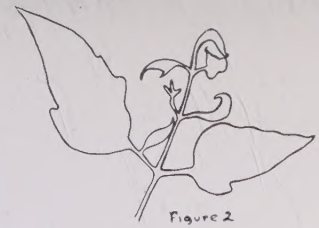


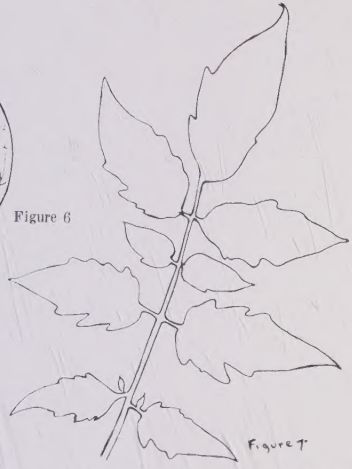
FIGURE 1. Leaf from June Pink tomato. Plant inoculated with severe mosaic type 1 on December 19, 1927. Drawing made on January 28, 1928. The distortion is very slight. See figure 2.

FIGURE 2. Leaf from a healthy June Pink tomato plant of same age and from a comparable position on the plant as leaf in figure 1.

FIGURE 3. Leaf from a June Pink tomato plant. Plant inoculated with severe mosaic type 2 December 19, 1927. Drawing made January 28, 1928. Of same age and from a comparable position on the plant as leaves in figures 1 and 2. Compare distortion with that in figure 1.







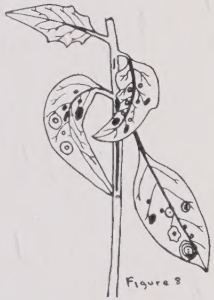


FIGURE 1. Leaf from a June Pink tomato plant inoculated with etch+ on December 19, 1927. Drawings made January 28, 1928. The narrow malformed leaves are typical of this type on tomato.

FIGURE 2. Leaf from a June Pink tomato plant inoculated with cucumber mosaic type 3+veinbanding on December 19, 1927. Drawing made January 28, 1928. Compare with figure 3.

FIGURE 3. Leaf from a June Pink tomato plant inoculated with cucumber mosaic type 1 on December 19, 1927. Drawing made January 28, 1928.

FIGURE 4. Early symptoms of ringspot on a cucumber leaf.

FIGURE 5. Leaf from same plant from which the one in figure 2 was taken. Drawing made on the same date. No normal leaflets developed.

FIGURE 6. Cotyledon of a cucumber plant showing early symptoms of ringspot. This plant inoculated January 19, 1925 and the drawing made January 27, 1928. The six solid dots are pin pricks.

FIGURE 7. Leaf from a June Pink tomato plant inoculated with etch on December 19, 1927. Drawing made January 28, 1928. Compare with healthy leaf and with figure 1.

FIGURE 8. Severe mosaic type 1 on Jimson showing the necrotic spots on pricked leaves and the streaks on the midveins and stem. Drawing made 10 days after inoculation.

FIGURE 9. Ring mosaic on June Pink tomato showing streaks on petioles and stem. Drawing made 23 days after inoculation.